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Exploring the mechanism of Lianhuaqingwen (LHQW) in treating chronic bronchitis based on network pharmacology and experimental validation

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Abstract

Background Lianhuaqingwen (LHQW) has been used in the treatment of chronic bronchitis, but the precise mechanism through which LHQW exhibits its anti-inflammatory effects in this context is not yet fully understood. The aim of this study was to investigate the active ingredients and signaling pathways responsible for LHQW's effectiveness in managing chronic bronchitis.

Methods The research leveraged the TCMSP database to determine the active compounds and drug targets of LHQW. In parallel, the GeneCards, DrugBank, and PharmGkb databases were used to uncover targets pertinent to chronic bronchitis. To discern the potential mechanisms by which LHQW's active ingredients might treat chronic bronchitis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed. Network pharmacology facilitated the construction of a drug-active ingredient-disease target network, aiding in forecasting the core targets for chronic bronchitis treatment by LHQW. Subsequently, molecular docking techniques alongside in vitro experiments were applied to confirm the interactions between the active ingredients and the primary targets.

Results A total of 157 active ingredients, 225 potential drug targets, and 594 bronchitis-related targets were derived from various databases. Following this, 76 potential gene targets were pinpointed by integrating drug and related targets. GO and KEGG enrichment analyses were employed to identify key pathways involved in LHQW's mechanism for treating chronic bronchitis. By constructing a protein–protein interaction (PPI) network for the 76 potential gene targets, four core targets (TNF, IL6, IFNG, and STAT3) were identified as primarily involved in responses to lipopolysaccharide, the TNF pathway, and the JAK-STAT pathway. Molecular docking results revealed a favorable affinity between multiple active ingredients of LHQW and the four core targets, suggesting that the therapeutic effects are mediated through the inhibition of inflammatory responses and signaling pathways. Interestingly, quercetin, an active ingredient of LHQW, was observed to bind to all four core targets simultaneously. Furthermore, cell experiment and western blot analysis indicated that both LHQW and quercetin exhibit anti-inflammatory effects by targeting the four core proteins and the JAK-STAT pathways.

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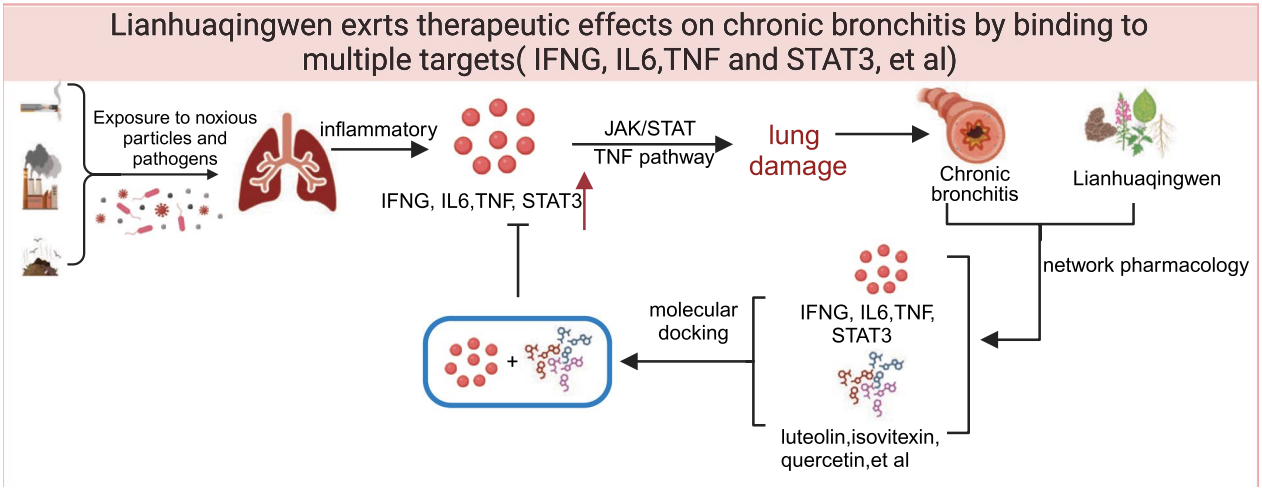
Conclusion This research emphasizes the diverse active ingredients, targets, channels, and pathways of LHQW in the treatment of chronic bronchitis, providing important perspectives for the creation of novel therapeutic drugs and clinical uses.

Keywords Lianhuaqingwen, Chronic bronchitis, Network pharmacology, Molecular docking

Graphical Abstract



Background

Chronic bronchitis, a common long-term respiratory illness with a high occurrence rate and prolonged duration, presents considerable challenges in its management. In China, the prevalence of chronic bronchitis in individuals above 50 years old stands at around 10%-15% [1]. Continuous presence of this condition may result in compromised lung function, heightened susceptibility to serious complications such as obstructive emphysema, pulmonary heart disease, and lung cancer, ultimately impacting both the health and quality of life of affected individuals [2–4]. The development of chronic bronchitis is a multifaceted process influenced by the intricate regulation of numerous genes and proteins. Present therapeutic approaches primarily concentrate on short-term infection management; however, they are associated with a high recurrence rate, adverse effects, and restricted long-term effectiveness. Hence, the pursuit of a safe and efficient treatment or drug target with minimal side effects has been a central area of research in recent times.

LHQW, an ancient form of traditional Chinese medicine that has been utilized for over 2000 years, is based on the medical practices of the Han, Ming, and Qing dynasties to treat human plague. A recent study involving 286 confirmed cases of COVID-19, conducted as a prospective multicenter open-label randomized controlled trial, discovered that when LHQW capsules were combined with standard treatment, there was an increased rate of recovery and a shorter duration of symptoms [5]. Another extensive double-blind randomized clinical trial on the effectiveness of LHQW in managing mild-to-moderate COVID-19 included 860 patients from 17 hospitals in China, Thailand, Vietnam, and the Philippines (trial registration number: ChiCTR2200056727). The results indicated that LHQW led to notable enhancements in clinical manifestations and decreased recuperation time for individuals with mild-to-moderate COVID-19 [6]. Additionally, studies have demonstrated that LHQW possesses anti-inflammatory properties by suppressing cytokine responses, which may help in addressing chronic bronchial inflammation and reducing lung injury [7]. Nevertheless, the precise mechanism by which LHQW operates is not yet fully understood due to its intricate composition.

Network pharmacology, as a multidisciplinary approach to research, plays a key role in understanding disease origins, pinpointing therapeutic goals, and uncovering the complex multi-target and multi-pathway mechanisms of traditional Chinese medicine. By creating molecular networks tailored to specific diseases, this methodology not only provides fresh perspectives and strategies for treating illnesses, but also allows for the

examination of how different active ingredients in traditional Chinese medicine interact synergistically and establishes connections between active ingredients and targets within networks. In the present investigation (see Fig. 1), a network pharmacology analysis identified four primary targets for chronic bronchitis treatment: tumor necrosis factor (TNF), interleukin 6 (IL6), interferon gamma (IFNG), and signal transducer and activator of transcription 3 (STAT3). Bioinformatics assessment demonstrated that these key targets participate in responses to lipopolysaccharide, the TNF signaling pathway, and the JAK-STAT signaling pathway, resulting in anti-inflammatory effects.

Methods

Active ingredients in LHQW

The active ingredients of LHQW were identified in Traditional Chinese Medicine Systems Pharmacology (TCMSP) by using keywords like "*Forsythia suspensa (Thunb.) Vahl*" (Lianqiao), *"Ephedra sinica Stapf*" (Zhimahuang), "*Lonicera japonica Thunb.*" (Jinyinhua), "*Prunus armeniaca var. armeniaca*" (Chaokuxingren), "*Isatis tinctoria L.*" (Banlangen), "*Pteris multifida Poir.*" (Mianmaguanzhong), "*Pogostemon Cablin (Blanco) Benth.*" (Guanghuoxiang), "*Houttuynia cordata Thunb."* (Yuxingcao), "*Rheum officinale Baill.*" (Dahuang), "*Mentha canadensis L.*" (Bohenao), and "*Glycyrrhiza uralensis Fisch.ex DC.*" (Gancao) as search terms [8] according to the stipulations of OB ≥ 30% and DL ≥ 0.18 [9]. Each plant name has been validated and referenced at http://www.theplantlist.org.

Pharmacokinetics analysis

The pharmacokinetic characteristics of the top 30 active ingredients were analyzed using the SwissADME tool (http://www.swissadme.ch/) [10], arranged according to the number of drug targets. These characteristics include physical and chemical properties such as molecular weight, the number of rotatable bonds, the count of hydrogen bond donors, and the quantity of hydrogen bond acceptors. Additionally, the analysis encompassed fat solubility, water solubility, gastrointestinal absorption (GI absorption), blood–brain barrier permeability (BBB permeant), P-glycoprotein substrates, and five distinct drug evaluation rules (Lipinski, Ghose, Veber, Egan, and Muegge).

Drug targets related to active ingredients

The TCMSP database was used to retrieve drug targets related to the active ingredients in LHQW. These potential drug targets were subsequently confirmed, standardized, and screened using UniProt (UniProt Consortium, 2015), after which they were identified as the relevant targets for the active ingredients in LHQW [9].

Screening of chronic bronchitis-related gene targets

Potential targets relevant to chronic bronchitis were identified through the GeneCards database [11], DrugBank database [12], and the PharmGkb database [13]. These identified genes were further verified and standardized via screening on UniProt (UniProt Consortium, 2015). The data from the three databases were integrated using R4.3.1 software, employing the Venn Diagram package, and duplicates were eliminated. This approach allowed for the finalization of gene targets associated with chronic bronchitis.

Construction and analysis of drug-active ingredient-disease target network

By using the Venn diagram tool, we identified the overlap in targets for LHQW and bronchitis to pinpoint potential drug targets. Next, we entered all ingredients and potential gene targets of LHQW into Cytoscape3.9.1 software to construct a network illustrating the connections between drugs, ingredients, and disease targets for treating chronic bronchitis. To analyze the relationships between active ingredients and gene targets, we conducted topological analysis with CytoNCA.

GO and KEGG pathway enrichment analysis

The software R-language (R4.3.1) was utilized with packages 'clusterProfiler', 'org.Hs.eg.db', 'enrichplot', and 'ggplot2' to perform pathway analysis for GO and KEGG [14]. Information for GO and KEGG was obtained using 'org.Hs.eg.db'. Enrichment analysis was conducted with a p-value cutoff of 0.05 and a q-value cutoff of 0.05.

Construction and analysis of the PPI network

Utilizing the STRING database (https://string-db.org/, version 12.0), the potential interactions of targets were examined, with the organism specified as 'Homo sapiens' and a confidence score threshold ≥ 0.900 [15]. Cytoscape 3.9.1 software was employed to visualize the PPI network. Topological analysis was performed to pinpoint the central targets in the network.

Cluster module analysis of the potential targets

We utilized the Metascape database (https://metascape.org/) for conducting cluster analysis to examine the roles of 76 potential pharmacodynamic genes. The criteria for pathway and process enrichment filtering required a significance level of $p < 0.01$, with a minimum of 3 genes and an enrichment of at least 1.5. Meanwhile, the filtering parameters for protein–protein interaction enrichment were set at 11% physical core.

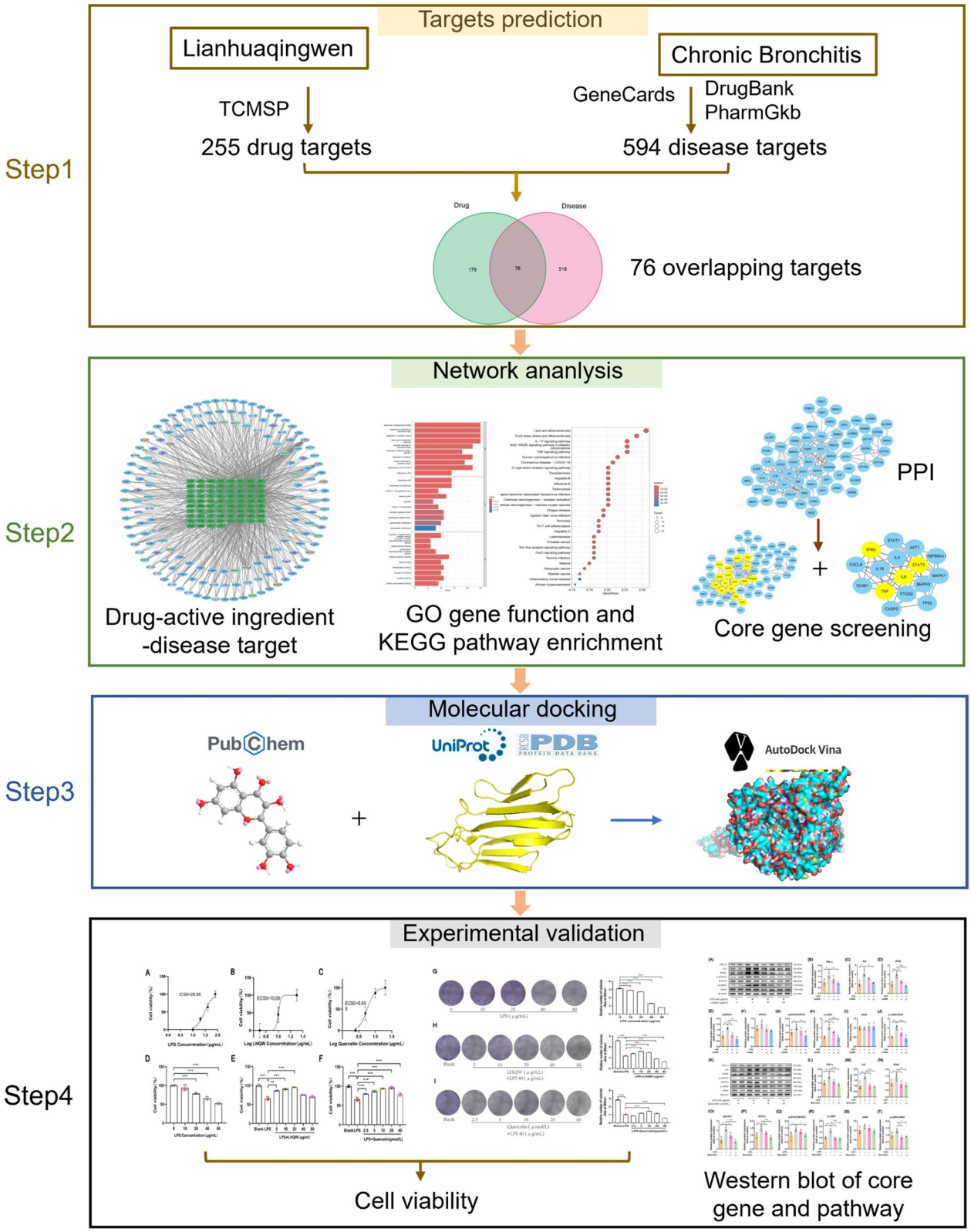


Fig. 1 Flow chart showing the experimental design of this study

Active ingredients and core targets interaction analysis

The crystal structures of the main targets were obtained from the RCSB PDB database (https://www.rcsb.org/), and the compositions of the key ingredients were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Before conducting molecular docking, structural water molecules and ligands were removed using the Pymol software. Following this, AutoDockTools was used to add polar hydrogen atoms and aid in molecular docking [16]. Stability and binding affinity were evaluated based on the lowest binding free energy detected in the interaction between the key ingredient and main target.

Cell culture and intervention

The human bronchial epithelial (16HBE) cells derived from Guangzhou Medical University in China, were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 nM L-glutamine, and 1% penicillin–streptomycin. Afterwards, the cells were treated with LPS (Beyotime, Biotechnology, Shanghai, China) [17], LHQW (Lot No. B2301053H, Yiling Pharmaceutical Co. Ltd., Shijiazhuang, China) [7], and quercetin (HY-18085, MedChemExpress, NJ, USA) [18] at prescribed concentrations and durations as specified in the findings section. Subsequent to the incubation period, a series of experiments including MTT assay, colony formation assay, and western blot analysis were performed.

Thiazolyl blue (MTT) assay

The 16HBE cells were detached by employing trypsin and subsequently plated onto a 96-well dish at a cellular density of 1×10^3 cells per well. After an intervention period of 48 h, the cells were exposed to 10 µL of 5 mg/mL MTT solution for a duration of 4 h, and subsequently subjected to treatment with 100 µL of dimethyl sulfoxide solution (DMSO), adequately mixed, and incubated. The optical density (OD) values at 562 nm were gauged using a microplate reader.

Colony formation assay

The 16HBE cells were detached with trypsin and seeded at a density of 500 cells per well in a 6-well plate. The medium was refreshed every three days. Following a culture and intervention period of 10 days, the cells were treated with crystal violet staining solution and DMSO. The relative colony count was then quantified using a microplate reader, measuring the optical density at 500 nm.

Protein isolation and western blot

The 16HBE cells underwent lysis in RIPA Lysis Buffer (Beyotime) containing protease and phosphatase inhibitor cocktails to analyze proteins. Protein levels were assessed using a PierceTM BCA Protein Assay Kit (Thermo Fisher Scientific). After separating equal amounts of protein with 12% SDS-PAGE, they were transferred onto PVDF membranes (ISEQ00010; Millipore, Burlington, MA, USA) and blocked using 5% non-fat milk. An array of specific antibodies including TNF-α (60291-1-lg, Protein Tech Group), IL6 (AF7236, Beyotime), IFNG (15365-1-AP, Protein Tech Group), STAT3 (cat. #9139; Cell Signaling Technology, Danvers, MA, USA), phosphoSTAT3 (#9145, Cell Signaling Technology), JAK (AF1489, Beyotime), phosphoJAK (AF1486, Beyotime), or β-actin (cat. # 60008-1-Ig, ProteinTech Group, Rosemont, IL, USA) were utilized for overnight incubation at 4 °C. The membranes were then exposed to a secondary antibody (peroxidase conjugated Affinipure Goat Anti Mouse/Rabbit IgG, ProteinTech Group) for 2 h at room temperature. The detection of immunoreactive bands was achieved using enhanced chemiluminescence (Thermo Fisher Scientific) and Tanon4600 automatic chemiluminescence image analysis system (Tanon, Shanghai, China). The subsequent analysis of quantitative data was conducted using ImageJ® software.

Statistical analysis

Statistical analyses were carried out using SPSS 26.0 software (IBM, Armonk, NY, USA) and GraphPad Prism software (version 10.0, La Jolla, CA, USA). The normality of the data was evaluated through Kolmogorov–Smirnov and Shapiro–Wilk tests, and the homogeneity of variance was assessed with Levene's test. One-way ANOVA with Bonferroni correction was utilized to compare three or more groups. The Kruskal–Wallis test was employed for variables that did not follow a normal distribution ($P < 0.05$). Findings were reported as mean ± standard deviation.

Results

Potential drug targets and pharmacokinetic properties of active ingredients in LHQW

LHQW is made up of 13 components, 11 of which are ingredients from traditional Chinese medicine. According to data from the TCMSP database, a total of 157 active components were found in LHQW, with 19 from *Forsythia suspensa* (Lianqiao), 22 from *Ephedra sinica Stapf* (Zhimahuang), 17 from *Lonicera japonica Thunb* (Jinyinhua), 16 from *Prunus armeniaca var. armeniaca* (Chaokuxingren), 28 from *Isatis tinctoria L.* (Banlangen), 2 from *Pteris multifida Poir.* (Mianmaguanzhong), 9 from *Pogostemon Cablin (Blanco) Benth.* (Guanghuoxiang), 5 from *Houttuynia cordata Thunb.* (Yuxingcao), 10 from *Rheum officinale Baill.* (Dahuang), 9 from *Mentha canadensis L.* (Bohenao), and 88 from *Glycyrrhiza*

uralensis Fisch. ex DC. (Gancao) (refer to Table 1). Upon removing duplicate targets, a total of 255 potential drug targets linked to the 157 active components were identified through the utilization of the TCMSP and UniProt databases.

SwissADME was utilized to predict the pharmacokinetic parameters of the active ingredients in LHQW for the treatment of chronic bronchitis. The selected properties included 'The Rule of 5', gastrointestinal absorption, blood–brain barrier (BBB) permeability, and P-glycoprotein substrates of the drug type (see Table 2). 'The Rule of 5' posits that poor absorption or permeation is more likely when there are more than five hydrogen bond donors, more than ten hydrogen bond acceptors, when the molecular weight (MWT) exceeds 500, or when the calculated Log P (CLogP) is greater than 5 (or MlogP > 4.15) [19]. Therefore, the results indicate that the active ingredients of LHQW exhibited a favorable gastrointestinal absorption and bioavailability.

Construction of drug-active ingredient-disease target network

606 targets related to bronchitis were identified through screening GeneCards, DrugBank, and PharmGkb databases. After eliminating duplicates, 594 targets were obtained (Fig. 2A). A comparison between the potential drug targets of LHQW and chronic bronchitis targets revealed 76 shared gene targets. These shared targets were considered potential gene targets for the 157 active ingredients of LHQW in treating chronic bronchitis (Fig. 2B). A visual representation of the network connecting drug-active ingredients-disease targets for LHQW in chronic bronchitis treatment demonstrated the interconnected relationships (Fig. 2C). This study emphasized the complex, multi-target approach of LHQW in addressing chronic bronchitis.

Table 1 Active ingredients of LHQW

Traditional name	Scientific name of the plant	active ingredients
Lianqiao	*Forsythia suspensa (Thunb.) Vahl*	19
Zhimahuang	*Ephedra sinica Stapf*	22
Jinyinhua	*Lonicera japonica Thunb*	17
Chaokuxingren	*Prunus armeniaca var. armeniaca*	16
Banlangen	*Isatis tinctoria L*	28
Mianmaguanzhong	*Pteris multifida Poir*	2
Guanghuoxiang	*Pogostemon Cablin (Blanco) Benth*	9
Yuxingcao	*Houttuynia cordata Thunb*	5
Dahuang	*Rheum officinale Baill*	10
Bohenao	*Mentha canadensis L*	9
Gancao	*Glycyrrhiza uralensis Fisch.ex DC*	88

GO and KEGG analysis of the LHQW treat on chronic bronchitis

Following the comparison and analysis of the GO functions and KEGG pathways for 76 potential gene targets of LHQW in treating chronic bronchitis, a detailed visualization analysis was performed on the top 10 items within biological processes (BP), cellular components (CC), and molecular functions (MF). This analysis is depicted in Fig. 3A. The findings revealed that gene targets showed substantial enrichment in BP terms such as 'response to lipopolysaccharide,' 'response to molecule of bacterial origin,' 'response to oxidative stress,' 'response to xenobiotic stimulus,' and 'cellular response to chemical stress.' Regarding CC, significant terms included 'membrane raft,' 'membrane microdomain,' 'vesicle lumen,' 'secretory lumen,' and 'cytoplasmic vesicle lumen.' As for MF, notable terms were 'G protein-coupled amine activity,' 'cytokine receptor binding,' 'heme binding,' 'phosphatase binding,' and 'cytokine binding.' Additionally, a visualization analysis of the top 30 KEGG signaling pathways, illustrated in Fig. 3B, indicated enrichment in pathways such as 'Lipid and atherosclerosis,' 'Fluid shear stress and atherosclerosis,' 'IL-17 signaling pathway,' 'AGE-RAGE signaling pathway in diabetic complications,' 'TNF signaling pathway,' 'Human cytomegalovirus infection,' and 'Coronavirus disease-COVID-19.'

Cluster module analysis and PPI network of LHQW in the treatment of chronic bronchitis target

Cluster module analysis of 76 potential pharmacodynamic target genes using the Matescape database was con, with a significance level of $p < 0.01$ and a minimum of three genes per cluster. This analysis yielded five cluster modules (Fig. 4A). Functional analysis of the targets within each cluster module revealed distinct association (Fig. 4B): MCODE1 was linked to signaling by interleukins, cytokine signaling in immune system, and IL-17 signaling pathway; MCODE2 was linked to spinal cord injury, regulation of small molecule process, and hepatitis C and hepatocellular carcinoma; MCODE3 was linked to monoamine GPCRs, amine ligand-binding receptors, and adrenaline signaling through alpha-2 adrenergic receptor; MCODE4 was linked to estrogen metabolism, chemical carcinogenesis-DNA adducts, and metabolism of xenobiotics by cytochrome P450; and MCODE5 was linked to DNA IR double strand breaks and cellular response via ATM, response to inorganic substance, and response to ionizing radiation.

After eliminating non-interacting proteins, the analysis of the PPI network displayed 66 nodes and 197 edges (Fig. 4C). A subsequent twice topological analysis (Fig. 4D, E) pointed four proteins—TNF, IL6, IFNG,

Table 2 Pharmacokinetic profiles of top 30 active ingredients of LHQW.

Active ingredients	Physicochemical properties of drugs							Pharmacokinetics			Generic prediction					
	MW (g/mol)	Rotatable bonds	H-bond acceptors	H-bond donors	XLOGP3	ESOL Log S	Ali Log S	GI absorption	BBB permeant	Pgp substrate	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Quercetin	302.24	1	7	5	1.54	− 3.16	− 3.91	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Kaempferol	286.24	1	6	4	1.9	− 3.31	− 3.86	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Luteolin	286.24	1	6	4	2.53	− 3.71	− 4.51	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Naringenin	272.25	1	5	3	2.52	− 3.49	− 3.99	High	No	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Glabridin	324.37	1	4	2	3.89	− 4.61	− 4.83	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Acacetin	284.26	2	5	2	3.35	− 4.14	− 4.71	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Aloe-emodin	270.24	1	5	3	1.82	− 3.04	− 3.43	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Wogonin	284.26	2	5	2	3.49	− 4.23	− 4.85	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Genkwanin	284.26	2	5	2	3.35	− 4.14	− 4.71	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
7-methoxy-2-methyl isoflavone	311.29	3	5	0	3.26	− 4.14	− 4.72	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Licochalcone B	286.28	4	5	3	2.6	− 3.41	− 4.08	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Formononetin	268.26	2	4	1	2.8	− 3.73	− 3.71	High	Yes	No	Yes	Yes	Yes	Yes	Yes	0.55
Licochalcone a	338.4	6	4	2	4.9	− 4.98	− 6.04	High	Yes	No	Yes	Yes	Yes	Yes	Yes	0.55
Isorhamnetin	316.26	2	7	4	1.87	− 3.36	− 4.02	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Irisolidone	314.29	3	6	2	2.96	− 3.97	− 4.49	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
Medicarpin	270.28	1	4	1	2.77	− 3.64	− 3.43	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Vestitol	272.3	2	4	2	2.94	− 3.69	− 3.84	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Shinptero-carpin	322.35	0	4	1	3.56	− 4.45	− 4.25	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
l-SPD	327.37	2	5	2	2.59	− 3.74	− 3.54	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Licoagro-carpin	338.4	3	4	1	4.54	− 4.96	− 5.27	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Glypallichal-cone	284.31	5	4	1	3.28	− 3.76	− 4.13	High	Yes	No	Yes	Yes	Yes	Yes	Yes	0.55
3′-methox-yglabridin	354.4	2	5	2	3.86	− 4.68	− 4.99	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
3′-hydroxy-4′-O-methyl-glabridin	354.4	2	5	2	3.86	− 4.68	− 4.99	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
1-methoxy-phaseollidin	354.4	3	5	2	4.18	− 4.81	− 5.32	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55

Table 2 (continued)

Active ingredients	Physicochemical properties of drugs							Pharmacokinetics			Generic prediction					
	MW (g/mol)	Rotatable bonds	H-bond acceptors	H-bond donors	XLOGP3	ESOL Log S	Ali Log S	GI absorption	BBB permeant	Pgp substrate	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
phaseol	336.34	2	5	2	4.69	− 5.25	− 6.18	High	No	No	Yes	Yes	Yes	Yes	Yes	0.55
bicuculline	367.35	1	7	0	2.61	− 4.02	− 3.66	High	Yes	No	Yes	Yes	Yes	Yes	Yes	0.55
7-ace-toxy-2– meth-ylisoflavone	294.3	3	4	0	3.22	− 4.03	− 4.08	High	Yes	No	Yes	Yes	Yes	Yes	Yes	0.55
Estrone	270.37	0	2	1	3.13	− 3.71	− 3.58	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Phaseoliniso-flavan	324.37	1	4	2	3.89	− 4.61	− 4.83	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
Machiline	285.34	3	4	3	2.58	− 3.46	− 3.52	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55

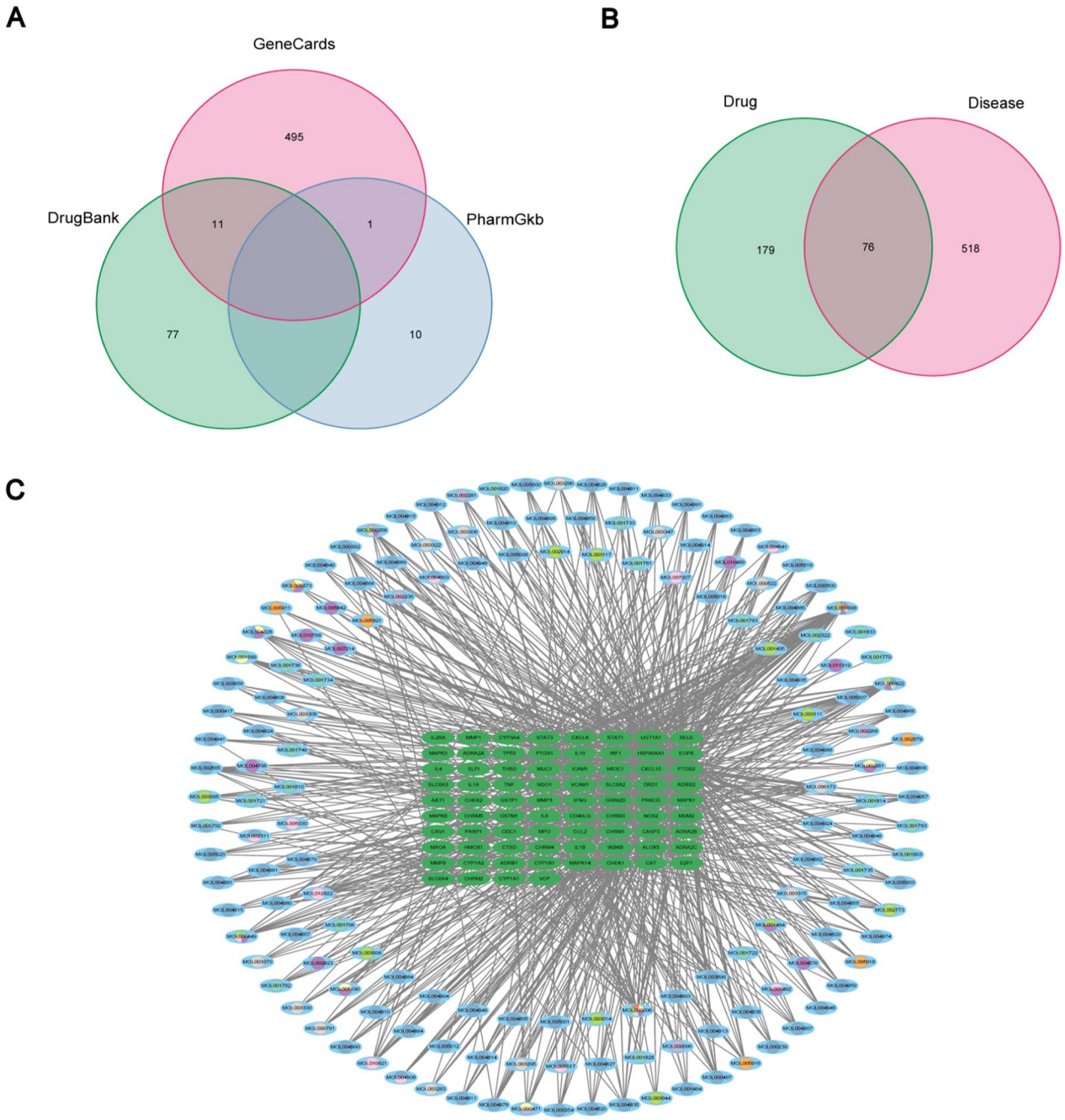


Fig. 2 Screening of potential gene targets for LHQW in the treatment of chronic bronchitis. **A** Diagram illustrating bronchitis-related targets using a Venn diagram. **B** Potential gene targets visualized through a Venn diagram. **C** Network illustrating the relationship between drug, active ingredient, and disease targets for LHQW. The central green node indicates potential targets for chronic bronchitis treatment, while the blue nodes signify active ingredients of LHQW

and STAT3 as having high degree, betweenness centrality, and closeness centrality within the PPI network. These pivotal proteins were recognized as essential in the treatment of chronic bronchitis with LHQW.

Validation by Molecular docking of active ingredients and core targets

The analysis of molecular docking was carried out to explore the possible binding of LHQW active components with core targets. TNF crystal structures (PDB

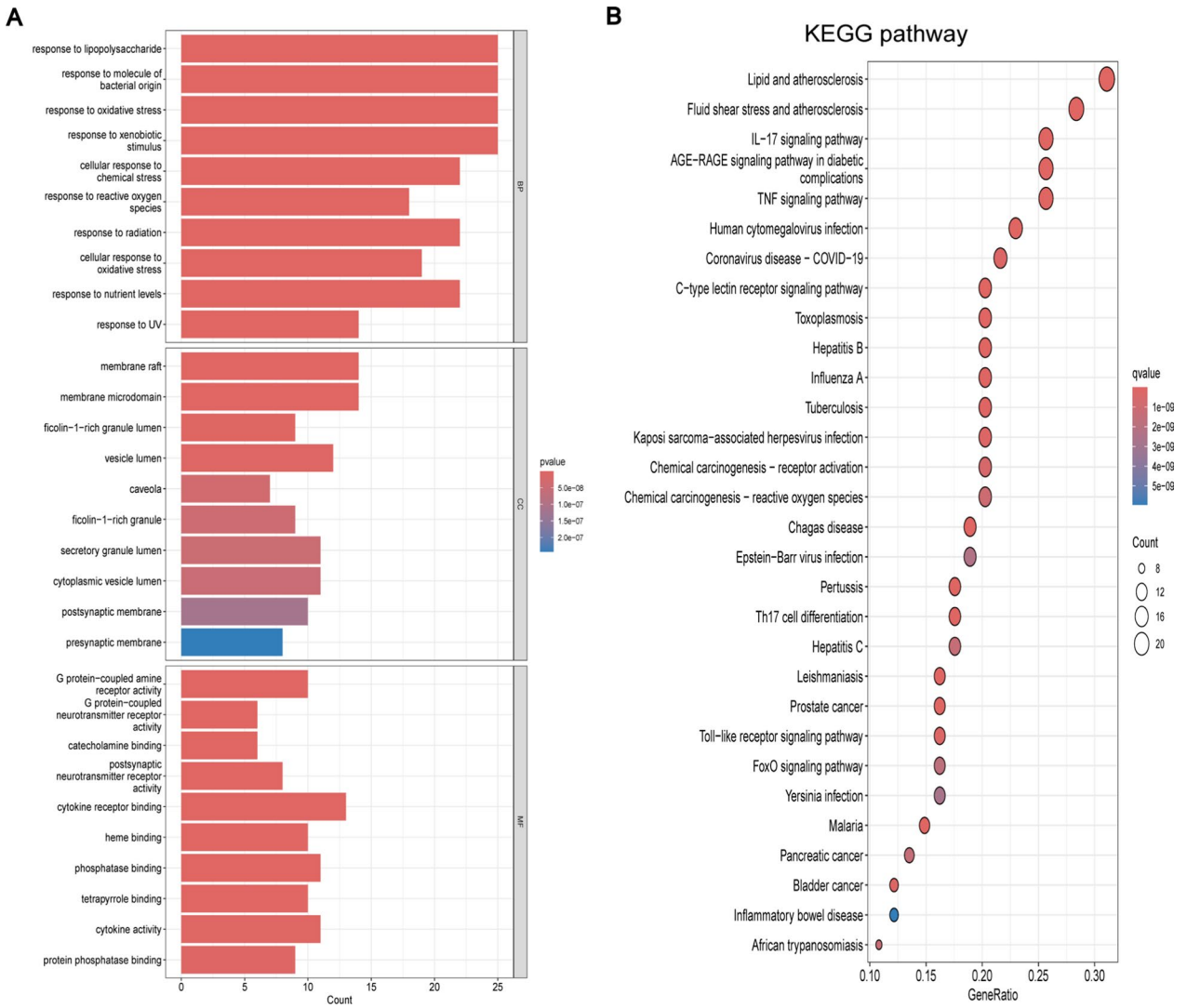
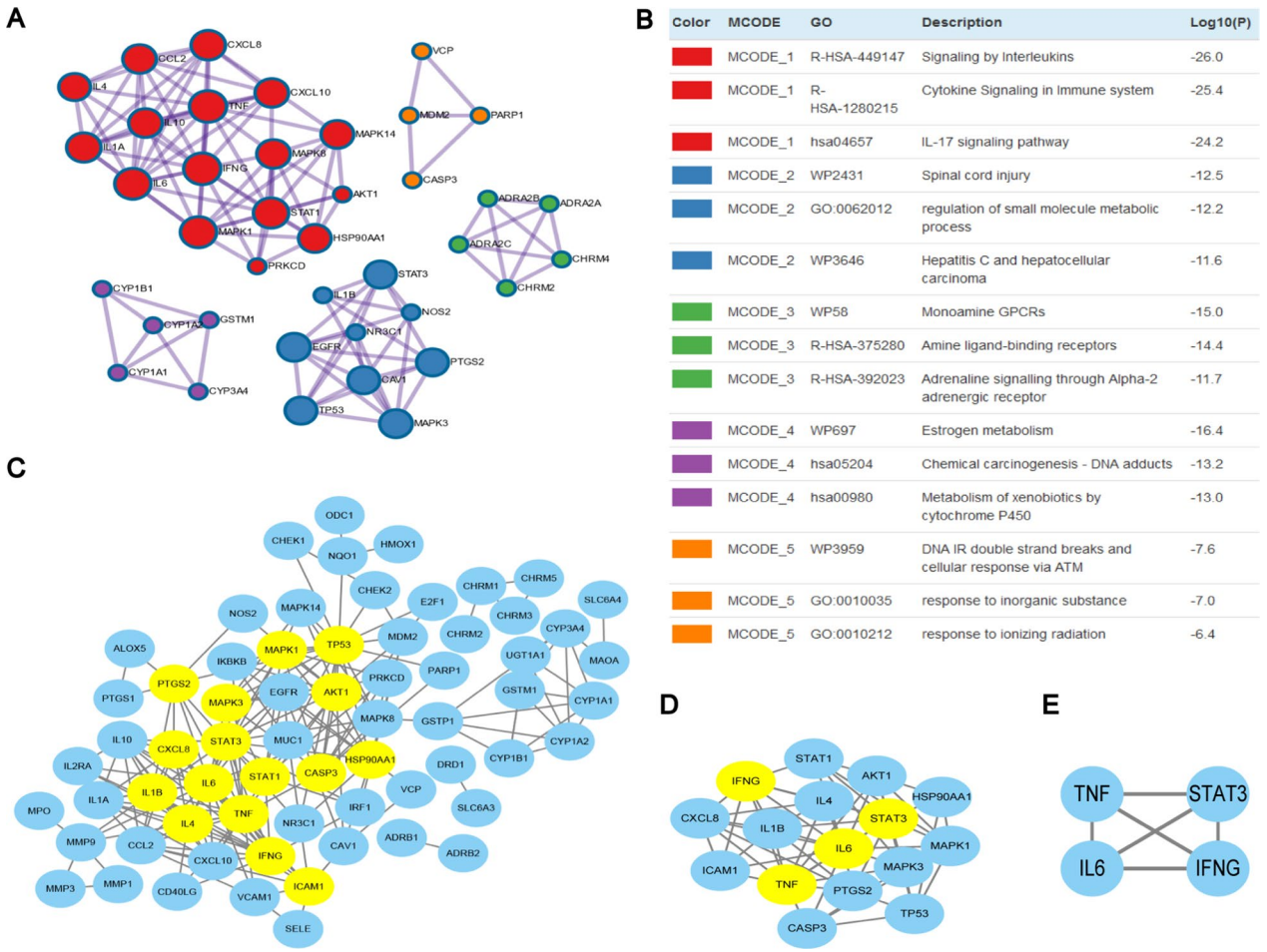


Fig. 3 Gene ontology (GO) enrichment and KEGG pathway analysis of potential gene targets for treating chronic bronchitis from active ingredients of LHQW. **A** The top 10 terms significantly enriched in biological processes (BPs), the top 10 terms significantly enriched in cellular components (CCs), and the top 10 terms significantly enriched in molecular functions (MFs) for the potential treatment of chronic bronchitis using active compounds from LHQW. **B** The top 30 enriched KEGG pathways for potential targets in the treatment of chronic bronchitis with active ingredients from LHQW

ID: 2Q1M), IL6 (PDB ID: 1ALU), IFNG (PDB ID: 1EKU), and STAT3 (PDB ID: 6NJS) were acquired from the PDB repository. The docking process involved pairing the components with the core targets, utilizing a binding energy threshold of − 5.0 kcal/mol for evaluating affinity levels. The findings illustrated in Fig. 5A–M presented the interactions between ligands and receptors. TNF exhibited strong affinity with quercetin, luteolin, wogonin, aloe-emodin, irisolidone, isovitexin, and kaempferol. Quercetin, luteolin, and wogonin demonstrated good affinity with IL6. IFNG showed good affinity with quercetin and luteolin, while STAT3 displayed strong interaction with quercetin. The binding energies for these connections were depicted in Fig. 5N, indicating varying levels of affinity. Quercetin emerged as a promising candidate due to its robust binding to all four core targets, hinting at its potential efficacy in addressing chronic bronchitis within LHQW (Fig. 5O). As is shown in Fig. 5A, the quercetin-TNF complex had one or two conventional hydrogen bonds with ASN120 and ARG116. Similarly, the quercetin-IL6 complex had two conventional hydrogen bonds with GLN175 and ARG179 (Fig. 5H). In the case of the



Color	MCODE	GO	Description	Log10(P)
	MCODE_1	R-HSA-449147	Signaling by Interleukins	-26.0
	MCODE_1	R-HSA-1280215	Cytokine Signaling in Immune system	-25.4
	MCODE_1	hsa04657	IL-17 signaling pathway	-24.2
	MCODE_2	WP2431	Spinal cord injury	-12.5
	MCODE_2	GO:0062012	regulation of small molecule metabolic process	-12.2
	MCODE_2	WP3646	Hepatitis C and hepatocellular carcinoma	-11.6
	MCODE_3	WP58	Monoamine GPCRs	-15.0
	MCODE_3	R-HSA-375280	Amine ligand-binding receptors	-14.4
	MCODE_3	R-HSA-392023	Adrenaline signalling through Alpha-2 adrenergic receptor	-11.7
	MCODE_4	WP697	Estrogen metabolism	-16.4
	MCODE_4	hsa05204	Chemical carcinogenesis - DNA adducts	-13.2
	MCODE_4	hsa00980	Metabolism of xenobiotics by cytochrome P450	-13.0
	MCODE_5	WP3959	DNA IR double strand breaks and cellular response via ATM	-7.6
	MCODE_5	GO:0010035	response to inorganic substance	-7.0
	MCODE_5	GO:0010212	response to ionizing radiation	-6.4

Fig. 4 Identification of core targets and cluster module analysis of LHQW in the treatment of chronic bronchitis. **A**, **B** Matescape database was utilized to conduct cluster module analysis of the 76 potential pharmacodynamic targets. The cluster modules were formed based on a significance level of $p < 0.01$ and comprised a minimum of three genes. **C** Interaction relationships were found among 66 out of the 76 potential target genes related to pharmacodynamics, depicted in a protein–protein interaction (PPI) network. The topological analysis of these potential pharmacological targets was carried out using Network Analyzer. The network displays the top 16 target genes ranked by degree, highlighted in yellow nodes. **D** The PPI network of 16 key pharmacological targets of LHQW for treating chronic bronchitis was derived from the initial topological analysis. The top four core targets in terms of degree ranking are represented by yellow nodes in the network. **E** The second topological analysis resulted in a PPI network of the four core targets of LHQW in the treatment of chronic bronchitis. Nodes in the network signify proteins, and the lines represent interactions between proteins

quercetin-IFNG complex, interactions were observed involving one or two conventional hydrogen bonds with PRO333, GLN168, GLN326, LYS58, and GLU324 (Fig. 5K). Moreover, the quercetin-STAT3 complex had one conventional hydrogen bonds with LEU436, LEU438, ASP369, LYS370, and THR440 (Fig. 5M). Notably, quercetin is found in multiple constituents of LHQW, including *Forsythia suspensa* (Lianqiao), (Mianmaguanzhong), *Ephedra sinica Stapf* (Zhimahuang), *Houttuynia cordata Thunb.* (Yuxingcao), *Glycyrrhiza uralensis Fisch. Ex DC.* (Gancao), *Pogostemon Cablin (Blanco) Benth.* (Guanghuoxiang), and *Lonicera japonica Thunb* (Jinyinhua) (Fig. 5P).

LHQW and quercetin reverses LPS-induced a reduction in cell viability in vitro

To investigate the impact of LPS, LHQW, and quercetin on the viability of 16HBE cells, two assays were carried out: MTT assay and colony formation assay were utilized to assess cell viability. Following a 24-h incubation with varying concentrations of LPS, the IC50 value was determined to be 28.86 μg/ml (see Fig. 6A). A comparison between the control group, treated with

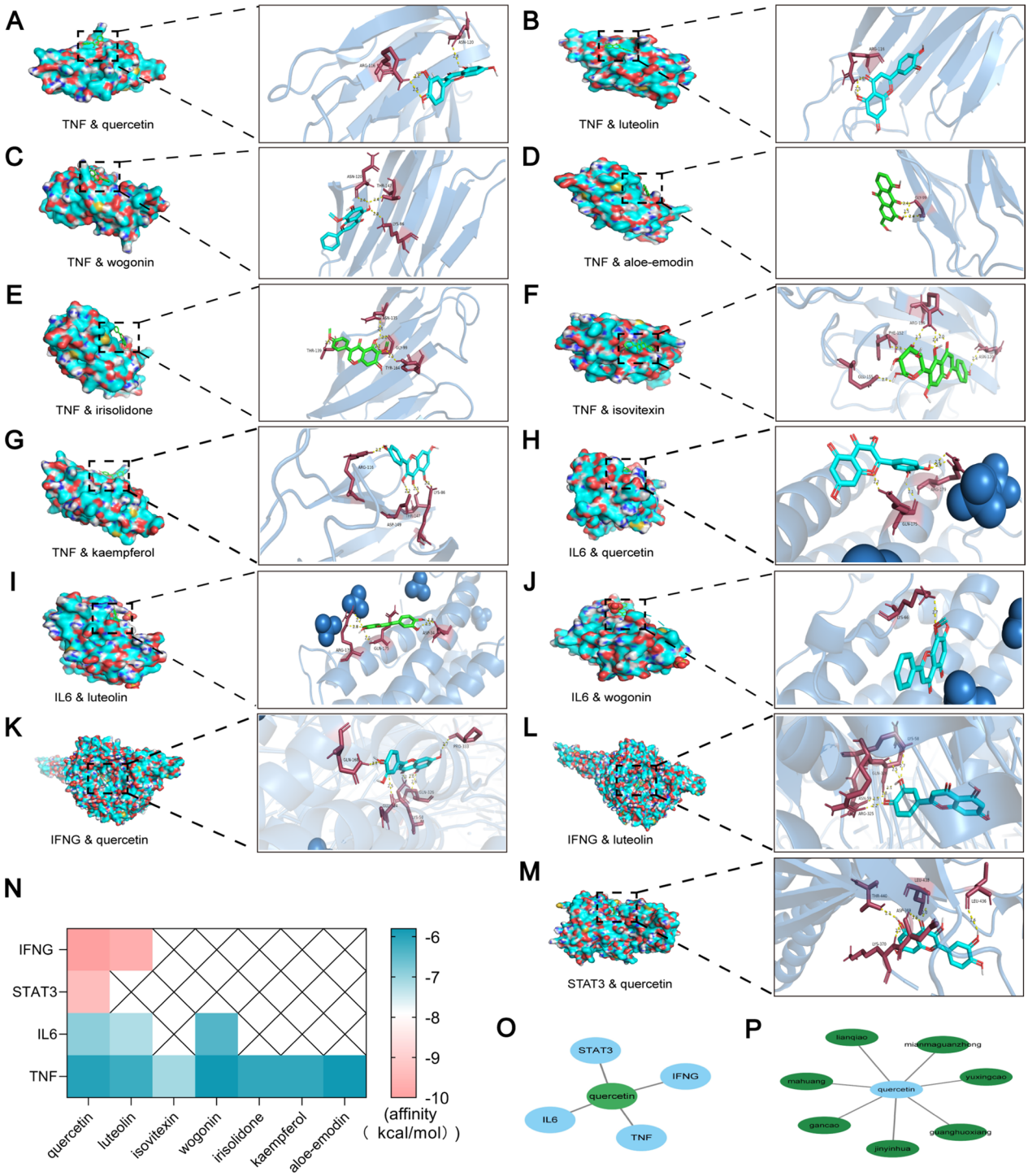


Fig. 5 Molecular docking of active ingredients and core targets. **A**–**M** The molecular docking results of active ingredients and core targets. **N** The docking score values for the interactions between active ingredients and core targets, where a lower score indicates a stronger binding affinity. **O** The regulatory network of quercetin's four core pharmacological targets in treating chronic bronchitis. **P** Chinese herbal medicine source of quercetin in LHQW

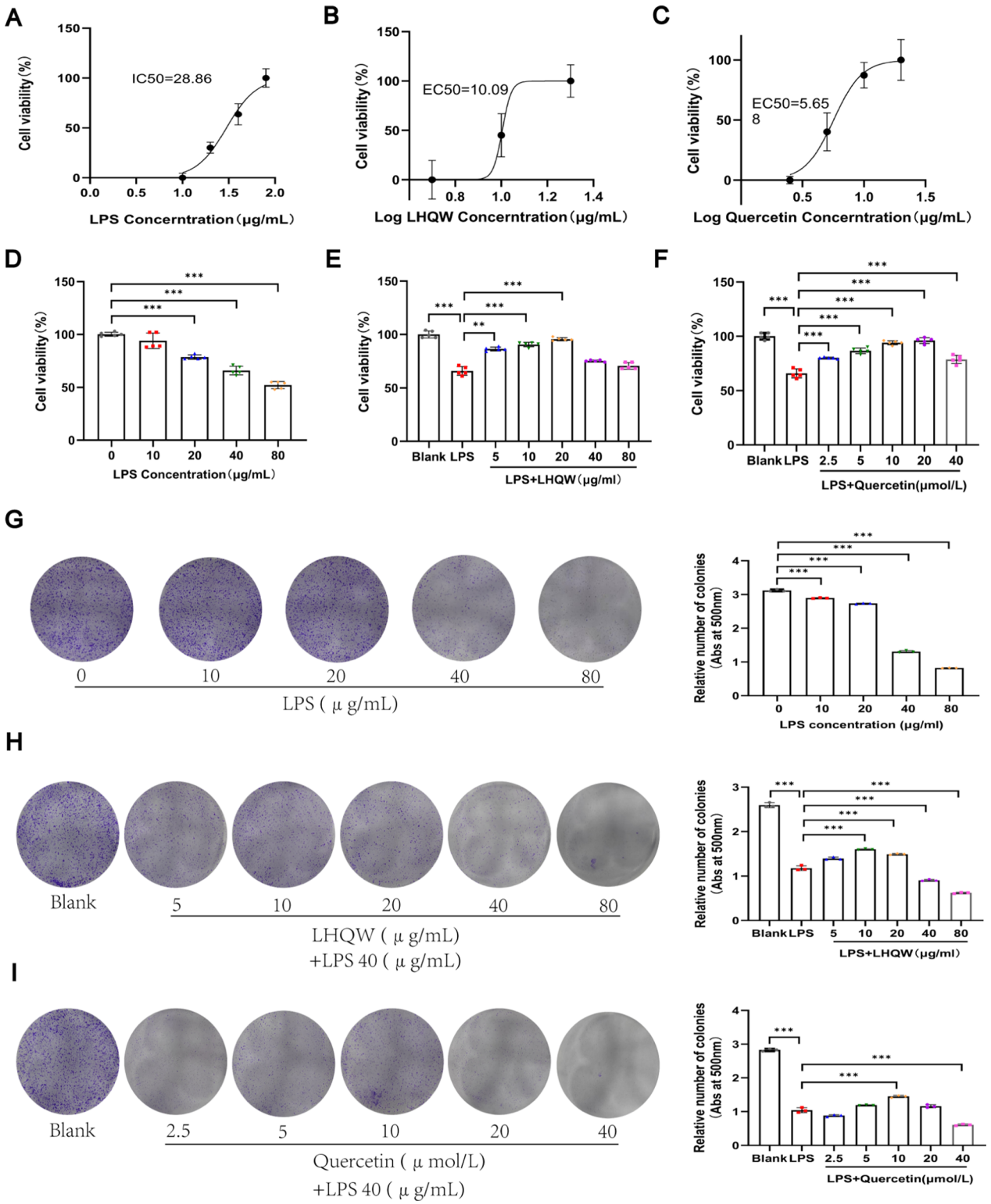


Fig. 6 LHQW and quercetin reverses LPS-induced a reduction in cell viability. **A** The IC_{50} in response to LPS using MTT assay was 28.86 μg/ml. **B** The EC_{50} in response to LHQW using MTT assay was 10.09 μg/ml. **C** The EC_{50} in response to quercetin using MTT assay was 5.65 μmol/L. **D**–**F** Cell Viability of 16HBE cells was assessed after 48 h of treatment with different concentrations of LPS, LHQW, and quercetin, n = 5 per group. **G**–**I** A colony formation assay was conducted with cells treated with different concentrations of LPS, LHQW, and quercetin. A representative of three experiments is shown, n = 3 per group. All data are presented as mean ± standard deviation, $^{**}p < 0.01$, $^{***}p < 0.001$

0 μg/ml of LPS, and the group treated with 10 μg/ml of LPS showed no significant change in cell viability (see Fig. 6D). However, colony formation revealed a decrease in the number of colonies in the 10 μg/ml LPS group (see Fig. 6G). Notably, cell viability exhibited a significant decrease upon treatment with LPS concentrations of 20, 40, and 80 μg/ml (see Fig. 6D). Moreover, the colony formation experiment corroborated a reduction in colony count (see Fig. 6G). These findings suggest that the optimal concentration of LPS to induce an inflammatory response in 16HBE cells is 40 μg/ml.

To establish the ideal concentrations of LHQW and quercetin, a range of LHQW concentrations (5, 10, 20, 40, 80 μg/ml) and quercetin concentrations (2.5, 5, 10, 20, 40 μmol/L) were tested on a 16HBE cell inflammation model induced by LPS (40 μg/mL). According to the MTT assay results, the EC50 values were determined to be 10.09 μg/ml for LHQW and 5.65 μmol/L for quercetin (Fig. 6B, C). Compared to the LPS control group, the LPS combined with LHQW at concentrations of 5 μg/ml, 10 μg/ml, and 20 μg/ml showed a marked increase in cell viability and colony formation, while concentrations of 40 μg/ml and 80 μg/ml of LHQW were associated with a reduction in colony numbers (Fig. 6E, H). Similarly, the groups treated with LPS and quercetin at 2.5 μmol/L, 5 μmol/L, and 10 μmol/L demonstrated a significant boost in cell activity and colony numbers (Fig. 6F, I). In contrast, the LPS + Quercetin (20 μmol/L) group exhibited a substantial rise in cell activity without a change in colony numbers, and the LPS + Quercetin (40 μmol/L) group showed enhanced activity yet reduced colony numbers (Fig. 6F, I). These observations indicate that the optimal concentrations are 10 μg/ml and 20 μg/ml for LHQW, and 5 μmol/L and 10 μmol/L for quercetin.

LHQW and quercetin reverses LPS-induced TNF, IL6, and IFNG expression, and the JAK-STAT pathway in 16HBE cells

To examine the effects of LHQW and quercetin on chronic bronchitis, 16HBE cells were treated with varying doses of LPS, LHQW, and quercetin. Analysis using Western blot demonstrated that LPS significantly raised TNFα, IL6, and IFNG concentrations in 16HBE cells, whereas LHQW and quercetin effectively decreased the expression of these proteins (seen in Fig. 7A–T). Furthermore, the activation of JAK2 (p-JAK2) and STAT3 (p-STAT3) was enhanced in LPS-induced 16HBE cells, yet LHQW and quercetin notably inhibited the levels of p-JAK2 and p-STAT3 (as depicted in Fig. 7A–T).

Discussion

Chronic respiratory diseases have become a major public health issue. Chronic bronchitis, a widespread long-term respiratory condition, is associated with numerous clinical outcomes. It serves as a prominent characteristic of chronic obstructive pulmonary disease (COPD), identified as a critical factor in both the progression and development of COPD [20]. An epidemiological study has shown that individuals with COPD and chronic bronchitis face a heightened risk of exacerbations and respiratory-related mortality [21]. The persistent cough linked to chronic bronchitis severely affects the quality of life, underscoring the importance of effective treatment approaches.

LHQW, a traditional Chinese herbal remedy, has been instrumental in treating the recent COVID-19 pandemic [5, 22]. Recent research has highlighted the remarkable healing benefits of LHQW in managing chronic bronchitis and acute COPD exacerbations [23, 24]. LHQW exhibits potent anti-inflammatory and immune-modulating properties, which can decrease the levels of inflammatory cytokines like TNF, IL17, and IL8, thus alleviating airway inflammation during COPD exacerbations [23]. Although LHQW has demonstrated clinical effectiveness in chronic bronchitis treatment, its underlying molecular mechanisms remain a mystery.

Utilizing network pharmacology, a network linking drugs, active ingredients, and disease targets can be constructed to explore the relationship between drugs and targets on both a systemic and molecular scale. This methodology is crucial for understanding the molecular mechanisms involved in drug treatment. The present investigation delves into the potential targets and molecular pathways targeted by LHQW for chronic bronchitis treatment, employing network pharmacology and molecular docking techniques. A thorough analysis revealed a grand total of 157 bioactive compounds and 255 therapeutic targets within LHQW. By cross-referencing data from GeneCards, DrugBank, and PharmGkb databases, a total of 594 genes linked to chronic bronchitis were identified. These genes were subsequently juxtaposed with the 255 targets associated with LHQW, leading to the identification of 76 potential gene targets for treating chronic bronchitis. Additional examination utilizing GO and KEGG pathways provided further elucidation on the functional roles of these targets.

Enrichment analysis indicated that LHQW may target therapeutic pathways for chronic bronchitis linked to BP, such as response to lipopolysaccharide, bacteria-derived molecules, and oxidative stress. Moreover, enrichment was evident in CC, including membrane raft, membrane microdomain, and vesicle cavity, as well as in MF, which included G protein-coupled neurotransmitter receptor

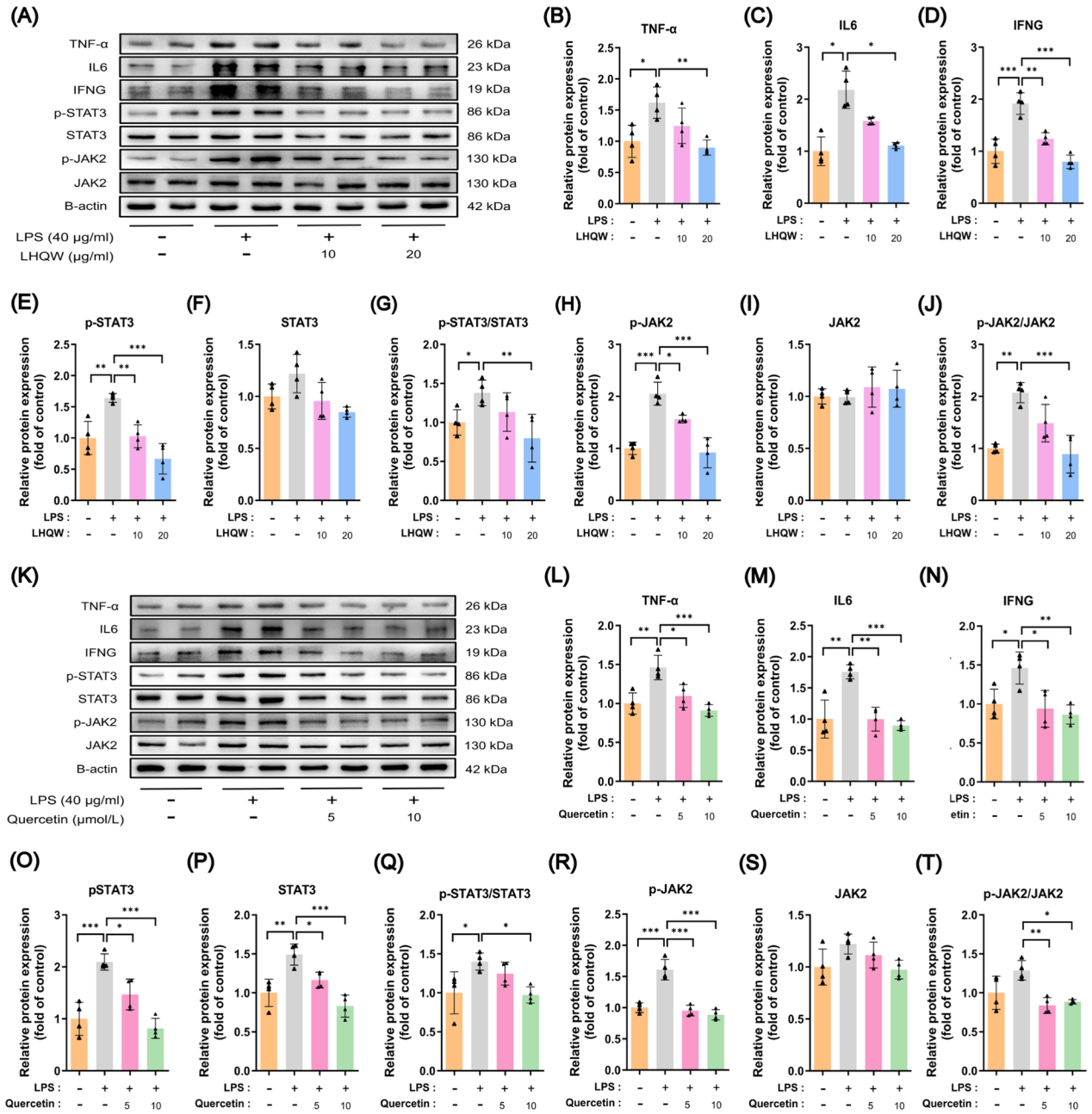


Fig. 7 LHQW and quercetin reverses LPS-induced TNF-α, IL6, and IFNG expression, and the JAK-STAT pathway in 16HBE cells. **A**–**J** Detection of TNF-α, IL6, IFNG, p-STAT3, STAT3, p-JAK2, and JAK2 protein expression in 16HBE following exposure to different doses of LPS and LHQW. **K**–**T** Analysis of TNF-α, IL6, IFNG, p-STAT3, STAT3, p-JAK2, and JAK2 protein levels in 16HBE treated with various doses of LPS and quercetin. Normalization of gene expression was normalized to *β-actin*, and the control group's protein levels were adjusted as "1". Data are means ± SD, n = 4 per group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

activity, cytokine receptor binding, and cytokine activity. Previous research has highlighted the significance of lipopolysaccharide in chronic bronchitis development, acting as a model for acute bronchitis, chronic bronchitis, and COPD in both animal and human studies [25–27]. Elevated oxidative stress has also been associated with inflammatory processes and the progression of chronic pulmonary diseases [28]. Additionally, KEGG pathway analysis demonstrated substantial enrichment in IL-17 and TNF signaling pathways, known for their roles in immune responses and inflammation in conditions like COPD and liver fibrosis [29–31]. Specifically, the

IL-17 pathway is linked to autoimmunity and pathogen defense, while the TNF-α pathway regulates pulmonary responses, inflammation, fibrosis [32], and apoptosis of alveolar epithelial cells [33]. These enrichment findings suggest that gene targets are mainly associated with chronic bronchitis development and progression.

In analyzing the PPI network of common targets for both LHQW and chronic bronchitis, pivotal core targets such as TNF, IL6, IFNG, and STAT3 were pinpointed following a dual topological analysis. Clustering module analysis indicated that these essential targets coagulated in MCODE1 and MCODE2, with predominant links to inflammation and immunity. The molecular docking results illustrated that LHQW's active components stably bind to these core targets, suggesting multiple therapeutic possibilities. These core targets are instrumental in cytokine production, TNF signaling, and JAK-STAT receptor signaling pathways, and interact to modulate immune responses. Extant studies have highlighted the roles of TNF, IL6, and IFNG in inflammation, and their interaction with STAT3 via the JAK-STAT signaling pathway to regulate the activity of immune cells and inflammatory responses [34–37]. Research conducted by Zhong et al. demonstrated that LHQW can mitigate inflammatory mediators such as TNF-α and IL6, subsequently ameliorating lung injury [7]. Our findings indicate that LHQW enhances cell viability and colony formation in LPS-induced 16HBE cells while concurrently reducing the expression levels of TNF-α, IL6, and IFNG and inhibiting the JAK-STAT pathway. Consequently, consistent with prior research [5, 6], LHQW shows multi-active ingredients interacting with multiple targets to treat chronic bronchitis.

Quercetin, a polyphenolic flavonoid present in *Forsythia suspensa* (Lianqiao), (Mianmaguanzhong), *Ephedra sinica Stapf* (Zhimahuang), *Houttuynia cordata Thunb.* (Yuxingcao), *Glycyrrhiza uralensis Fisch. exDC.* (Gancao), *Pogostemon Cablin (Blanco) Benth.* (Guanghuoxiang), and *Lonicera japonica Thunb* (Jinyinhua) in LHQW, displays a strong interaction with TNF, IL6, IFNG, and STAT3, highlighting its potential as a crucial active ingredient of LHQW. Recognized for its therapeutic benefits, quercetin suppresses inflammatory markers like TNF-α, IL6, IL8, and IFNG, along with indicators of oxidative stress such as LOX-1 and ROS [18]. In this investigation, akin to LHQW, quercetin can diminish the expression of TNF-α, IL6, and IFNG, while thwarting the JAK-STAT pathway. Furthermore, it is demonstrated that quercetin negatively modulates various signaling pathways, encompassing apoptosis, the NF-κB pathway, and the MAPK pathway [18]. Additionally, research has indicated that quercetin displays anti-cancer, antioxidant, antiviral, and anti-inflammatory properties, underscoring its usefulness in addressing cardiovascular conditions [38, 39], cancer [40], Alzheimer's disease [41, 42], and diabetes [43, 44]. Overall, both past investigations and our own results propose that quercetin carries clinical significance in managing chronic bronchitis.

LHQW demonstrates efficacy in the treatment of COVID-19, acute lung injury (ALI), and cancer. Researches have indicated that various active components of LHQW exhibit anti-SARS-CoV-2 and anti-inflammatory effects to alleviate associated clinical symptoms by targeting cytokines (IL6, IL10, TNF, IFNG, and MAPK) [45–47], immune responses [48], Akt1[49], ACE2 [50], and 3CLPro [51]. Four active components of LHQW (quercetin, luteolin, kaempferol, and wogonin) target genes such as AKT1, TP53, IL6, TNF, STAT3, and pathways related to apoptosis, contributing to the treatment of ALI [52]. Moreover, the top three compounds of LHQW (kaempferol, luteolin, and quercetin) exhibit a strong affinity for AKT1 and down-regulate the PI3K/AKT pathway to combat liver cancer [53]. Our study reveals that the active ingredients of LHQW target core genes (TNF, IL6, IFNG, and STAT3) and the JAK-STAT pathway to address chronic bronchitis. Due to its diverse active components and ability to interact with multiple targets, LHQW is involved in various transduction pathways and biological processes. Consequently, the therapeutic effects of LHQW on various diseases are not limited to specific targets, but rather arise from the active ingredients' capability to interact with multiple targets and participate in diverse pathways, particularly those associated with the development and progression of specific disease categories. Additionally, in the evaluation of security indexes, a large retrospective clinical study demonstrated LHQW exhibited a favorable safety profile without any drug-related adverse events [54]. A meta-analysis indicated that LHQW has a lower incidence of adverse reactions in clinical application compared to conventional drugs (Systematic Review Registration: CRD-42020224180) [55].

Conclusion

By employing network pharmacology and molecular docking analyses, TNF, IL6, IFNG, and STAT3 were identified as key targets with strong binding affinities to different active constituents of LHQW, underscoring its potential in treating chronic bronchitis. Quercetin in LHQW, in combination with other active ingredient demonstrates anti-inflammatory properties by suppressing TNF, IL6, and IFNG, as well as the JAK-STAT pathway. These results indicate that LHQW exhibits characteristics of numerous active components, targets, and pathways, offering a promising approach for managing chronic bronchitis.

Abbreviations

LHQW	Lianhuaqingwen
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PPI	Protein–protein interaction
COVID-19	Coronavirus disease 2019
COPD	Chronic obstructive pulmonary disease
ALI	Acute lung injury
TNF	Tumor necrosis factor
IL6	Interleukin 6
IFNG	Interferon gamma
STAT3	Signal transducer and activator of transcription 3
JAK	Janus kinase
16HBE	The human bronchial epithelial cells
DMSO	Dimethyl sulfoxide solution

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Author contributions
Lin SH: Conceptualization, Investigation, Data curation, Validation; Wang S: Investigation, Data curation, Validation; Jiang QP: Investigation, Data curation; Liu SY: Investigation, Data curation; Liu SJ: Conceptualization, Investigation, Validation, Funding acquisition, Writing—Original draft preparation; Cai TH: Conceptualization, Data curation, Validation, Funding acquisition, Writing—Reviewing and Editing, Supervision.

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Data availability
All the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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