

# *Exploring the Mechanism of Action of the Lung-Tonifying and Blood-Activating Formula in Treating the Stable Phase of Chronic Obstructive Pulmonary Disease Based on Network Pharmacology and Molecular Docking Technology*

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**Abstract:** This study employs a combined approach of network pharmacology and molecular docking techniques to systematically investigate the active constituents, target molecules, and molecular mechanisms underlying the therapeutic effects of the Lung-Nourishing and Blood-Activating Formula in treating chronic obstructive pulmonary disease (COPD). Through database screening using TCMSp alongside literature review, 82 active components and 946 drug targets were identified. A total of 1132 COPD-related targets were collected from GeneCards, NCBI, and DisGeNET databases, yielding 111 common targets upon intersection. Cytoscape was employed to construct an "active component-target" network, STRING analysed protein interactions, and GO and KEGG enrichment analyses were conducted using R. Results revealed 3,918 entries in the GO analysis, while KEGG analysis indicated 238 potential signalling pathways involved. Molecular docking further demonstrated strong binding affinity between key active components and core targets in the formula. Conclusions suggest that the Lung-Nourishing and Blood-Activating Formula may treat COPD through multi-component, multi-target, and multi-pathway synergistic effects. Its mechanism likely involves regulating signalling pathways such as JAK-STAT, NF-κB, PI3K/AKT, and MAPK to suppress inflammatory responses.

## 1. Introduction

Chronic obstructive pulmonary disease (COPD), characterised by persistent respiratory symptoms, is a heterogeneous pulmonary disorder defined by chronic respiratory symptoms<sup>[1]</sup>. Chronic obstructive pulmonary disease (COPD) induces chronic respiratory symptoms (dyspnoea,

cough, sputum production, acute exacerbations) and causes progressively worsening airflow limitation. Ranking third globally in mortality, it imposes substantial economic and health burdens worldwide<sup>[2]</sup>, representing a chronic disease with high incidence and mortality rates in China. The 2019 Global Burden of Disease, Injury, and Risk Factors Study reported COPD as the third leading cause of death in China. With accelerating population ageing and persistent exposure to environmental pollutants, the prevalence and incidence of COPD continue to rise, projected to peak among those aged 95 years and above<sup>[3]</sup>. It is evident that COPD remains a significant challenge for China's healthcare system, requiring both preventive measures and interventions to halt progression and prevent recurrence. Timely and effective management during stable phases can avert or reduce acute exacerbations, thereby slowing disease progression, lowering the risk of severe complications, and ultimately reducing the overall burden of COPD.

COPD primarily affects the lungs but also involves systemic responses; its pathogenesis remains complex and inconclusive. Modern medical approaches to COPD primarily focus on alleviating respiratory symptoms. Treatment typically involves inhaled short-acting bronchodilators (including  $\beta$ 2-agonists and anticholinergics) or oral theophylline. While these interventions can improve clinical symptoms to some extent, their effects on pulmonary and systemic inflammation, quality of life, and disease progression are limited<sup>[4]</sup>. Therefore, identifying alternative effective therapeutic approaches—preventing disease progression while managing concomitant conditions—offers significant benefits. Such interventions not only control symptoms but also promote disease management. In comparison, traditional Chinese medicine (TCM) demonstrates distinct advantages through its comprehensive, multi-faceted treatment of patient suffering. The Lung-Tonifying and Blood-Activating Formula was developed by modifying the Lung-Tonifying Granules based on clinical diagnostic experience. This study aims to further explore the intervention mechanisms for COPD using network pharmacology, while also providing a scientific rationale for the clinical application of the Lung-Tonifying and Blood-Activating Formula.

## 2. Materials and Methods

### 2.1 Establishment of the Formula Component and Target Databases.

The chemical constituents of each herb in the Lung-Tonifying and Blood-Activating Formula were collected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and various public literature sources. Active constituents were screened based on oral bioavailability (OB) exceeding 40% and drug-like properties (DL) above 0.18. Target names were standardised via the Uniprot platform.

### 2.2 Construction of Drug-Related Target Database and Disease-Associated Target Database.

Commencing target research from the TCMSP database, entries with prediction scores exceeding zero were selected to form a component target list for the Lung-tonifying and Blood-activating Formula. Using "Chronic obstructive pulmonary disease" as the keyword, corresponding targets were identified within the GeneCards database<sup>[5]</sup>, NCBI database<sup>[6]</sup>, and DisGeNET database<sup>[7]</sup>, filtering entries with association values exceeding 1. All data were consolidated, and a Venn diagram illustrating the intersection of drug, disease, and target data was generated using R 4.0.3.

### 2.3 Construction of Drug-Active Ingredient-Target Network.

After compiling the active components selected from the Lung-Nourishing and Blood-Activating Formula and their drug-target interactions, the data were imported into Cytoscape 3.10.0 to

construct a network diagram of drug-active component-target relationships.

#### **2.4 Construction of the Lung-Tonifying and Blood-Activating Formula and COPD Protein-Protein Interaction (PPI) Network**

Input the target proteins common to both drugs and the disease into the STRING database, setting the minimum interaction score threshold to 0.4. The resulting network was then processed using Cytoscape 3.10.0 to obtain the topological parameters of each node in the intersection network. Key proteins were screened based on their degree values.

#### **2.5 Gene Ontology (Go) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis**

Target proteins were analysed using relevant packages within R 4.0.3, with a P-value threshold of  $<0.05$ , yielding GO and KEGG enrichment analysis data.

#### **2.6 Molecular Docking**

Key targets were selected from the PPI network and combined with the top five active compounds for docking analysis. Three-dimensional structures of these active compounds were retrieved from the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP). Corresponding target protein structures were obtained from the Protein Data Bank (PDB). Active compounds were imported into AutoDockVina 1.2.3 alongside target proteins. Following fundamental processing steps, including dewatering, hydrogenation, and force field optimisation, molecular docking was performed. The resulting docking scores were recorded, and the docked structure images were saved.

### **3. Results**

#### **3.1 Composition and Target Mechanism of the Lung-Tonifying and Blood-Activating Formula**

Analysis revealed the Lung-Nourishing and Blood-Activating Formula comprises 14 herbal ingredients yielding 82 compounds, with 27 shared active constituents. These 82 compounds were analysed using the SEA database (<https://sea.bkslab.org/>), setting a p-value threshold of 0.05 as the screening criterion, and after removing duplicates, 701 targets were obtained. These were then predicted using the SuperPredict database (<https://prediction.charite.de/>), setting a probability threshold of 0.6 as the criterion. After removing duplicates, 392 targets were obtained. The results from both methods were merged, yielding a final total of 946 targets.

#### **3.2 COPD Disease Targets**

Using the keyword "Chronic Obstructive Pulmonary Disease" to search for COPD-related targets across three databases (GeneCards, NCBI, DisGeNET), 781 targets were identified in GeneCards, NCBI yielded 545, and DisGeNET displayed 23, totalling 1,349. After removing duplicates, 1,132 disease-associated targets were identified. These overlapped with the 946 targets from the Lung-Nourishing and Blood-Activating Formula, ultimately yielding 111 shared targets, as detailed in Figure 1.

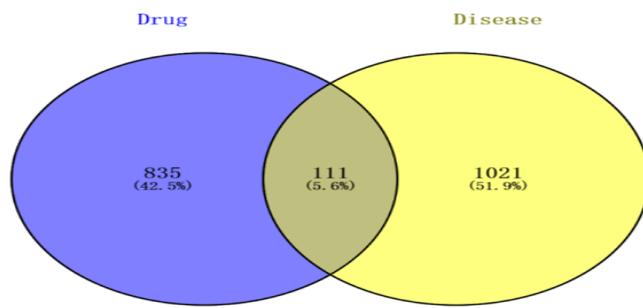


Figure 1: Screening results of COPD disease targets.

### 3.3 Construction of the "Drug-Active Ingredient-Target" Interaction Network

Using the Cytoscape 3.10.0 platform, the active components of the Lung-Nourishing and Blood-Activating Formula were integrated with the "drug-active ingredient-target" interaction network to form a comprehensive "drug-active ingredient-target" network. This clearly visualised the relationships among 82 active ingredients and 9 potential targets. The 82 potential targets were ranked by frequency of interaction: apigenin, demethoxyflavone, and subenin A from *Scutellaria baicalensis*; eupatorin from *Paeonia lactiflora*; (+)-1(R)-eupatorine from *Prunus armeniaca*. These constituents may represent key chemical entities within the Lung-Nourishing and Blood-Activating Formula for treating stable COPD. Overall analysis indicates the formula exhibits multi-constituent, multi-target characteristics, with its efficacy primarily manifested in lung-nourishing and blood-activating functions. This formulation possesses therapeutic effects through multiple constituents and multiple targets, as shown in Figure 2.

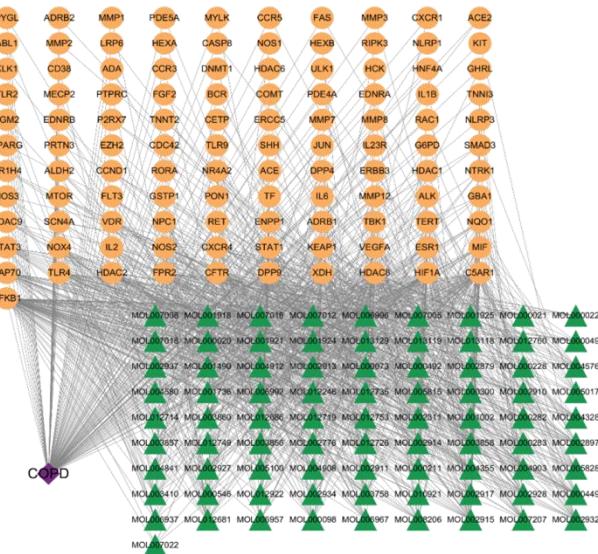


Figure 2: Component-Disease-Target Network Diagram Note: Green nodes represent targets, purple nodes represent diseases, and orange nodes represent compounds.

### 3.4 Construction of Protein-Protein Interaction Networks

Four PPI networks were constructed by inputting four shared targets into the STRING database, forming the 4PPI network. The entire network comprises 1,103 edges, reflecting connections between nodes. Using Cytoscape 3.10.0 software and sorting by degree values, the top five nodes

are: Interleukin (IL)-6, Interleukin-1 $\beta$ , Signal Transducer and Activator of Transcription (STAT) 3, JUN protein 1 $\alpha$ . These targets played crucial roles when the Lung-Nourishing and Blood-Activating Formula was employed to intervene in COPD. Detailed information can be found in Figures 3 and 4.

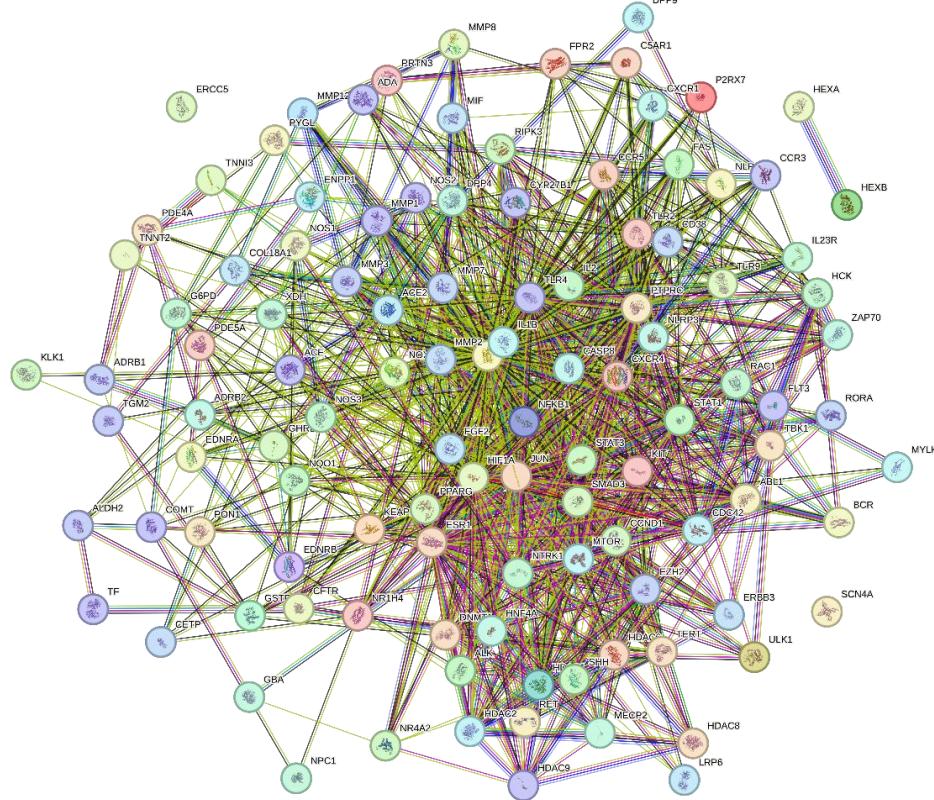


Figure 3: Construction of the PPI network.

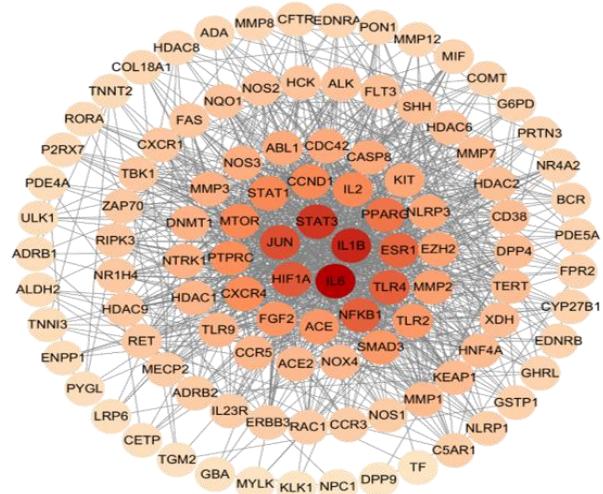


Figure 4: PPI Network Diagram Note: The inner circle depicts targets with higher degrees, i.e., key targets, namely IL-6, IL-1 $\beta$ , STAT3, JUN, and HIF1A (TOP5). These targets will be utilised for molecular docking.

### 3.5 Go and KEGG Enrichment Analysis

For the 111 target genes common to both the Lung-Nourishing and Blood-Activating Formula and COPD, GO annotation analysis was conducted across biological process (BP), cellular component (CC), and molecular function (MF) dimensions. Using the String database, entries with corrected P-values below 0.05 were filtered. This yielded 3,918 enriched entries in biological process, 455 in molecular function, 251 in cellular component, and 251 entries in cellular composition. Using R 4.0.3 with packages including clusterProfiler, enrichplot, and ggplot2, bubble plots were generated. A functional network diagram of KEGG pathway enrichment was constructed using relevant R 4.0.3 modules, primarily covering the atherosclerosis signalling pathway, Th17 signalling pathway, and COVID-19 signalling pathway. These results are presented in Figure 5 and Figure 6.

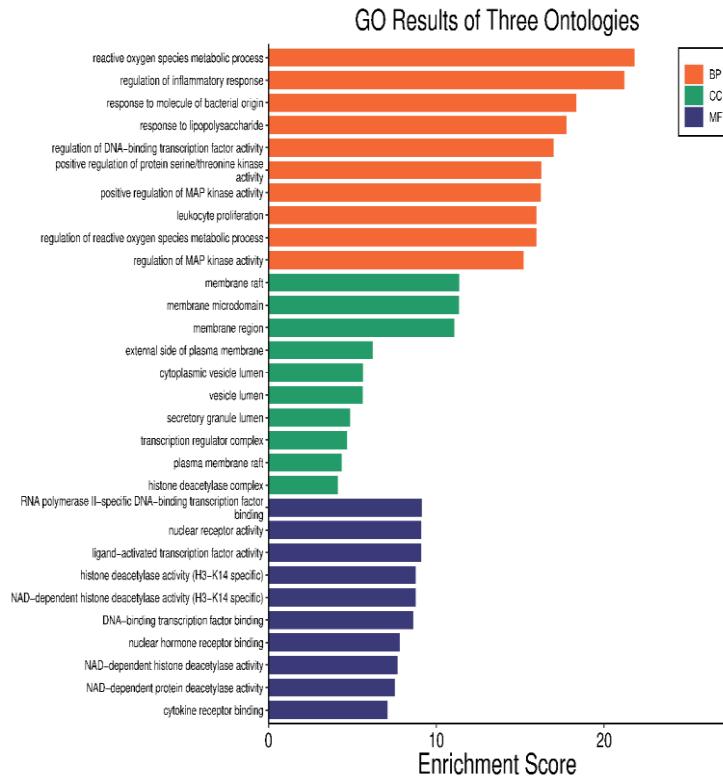


Figure 5: Bubble plot of GO enrichment analysis.

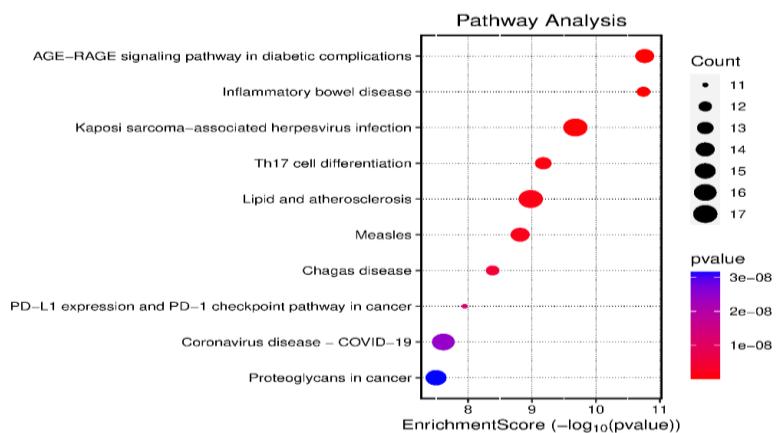


Figure 6: KEGG Pathway Enrichment Functional Network Diagram.

### 3.6 Molecular Docking and Validation

The top five key targets ranked by degree value from the PPI network were selected for molecular docking experiments with the active components of the drug. Results indicated that (+)-1(R)-Aconitine exhibited significant interaction with the IL-6 target, achieving the most prominent docking score. While dimethoxyflavone exhibited relatively high docking scores with IL-1 $\beta$ . Detailed data are presented in Figure 7. Analysis suggests that (+)-1(R)-Aconitine and dimethoxyflavone may play crucial roles in addressing COPD. The top two docking results and their two-dimensional structures are illustrated in Figures 7 and 8. Figure 8 illustrates the interaction between the small molecule IL1B and the MOL002932 protein. It is evident that MOL002932 forms hydrogen bonds with GLU-64, LYS-65, and ASN-7. This hydrogen bonding enhances the tightness of the protein-small molecule complex, constituting the primary binding mechanism between IL1B and MOL002932. Figure 9 depicts the interaction between the small molecule MOL007207 and the IL6 protein. The figure indicates that MOL007207 forms hydrogen bonds with SER-168, LEU-63, and GLU-171 of the IL6 protein. This binding pattern enhances the compactness between the small molecule and the protein. Concurrently, hydrophobic interactions exist with LEU-63, GLU-171, and LYS-65, providing strong van der Waals forces between the molecules.

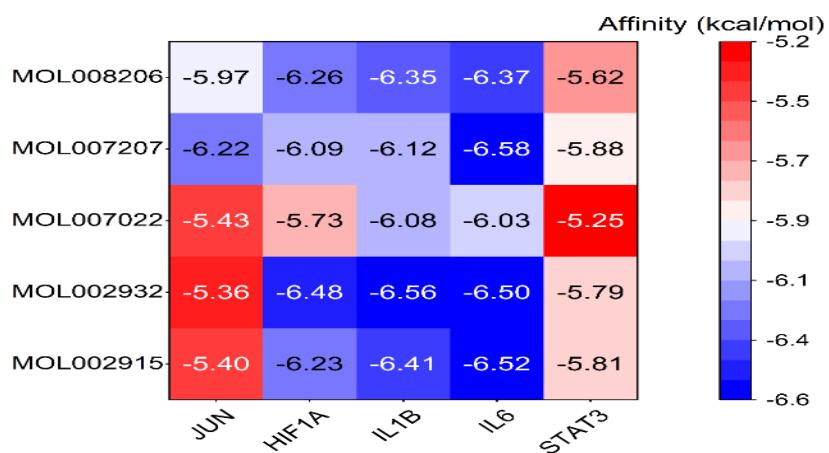


Figure 7: Alignment Scoring Heatmap Note: Darker colours indicate better alignment quality.

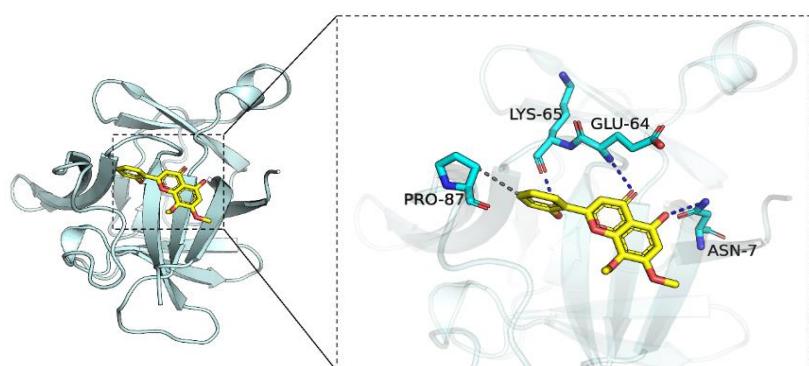


Figure 8: Interaction between small molecule IL-1 $\beta$  and MOL002932 protein.

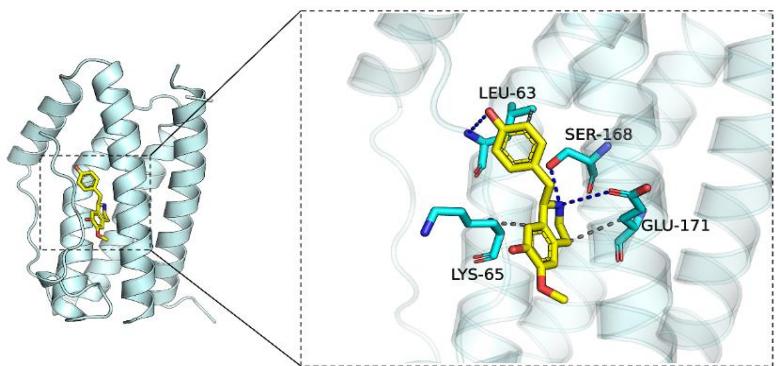


Figure 9: Interaction between small molecule MOL007207 and IL-6 protein.

#### 4. Discussion

Traditional Chinese medicine classifies chronic obstructive pulmonary disease (COPD) under symptoms such as "pulmonary distension", "coughing", "wheezing syndrome", and "pulmonary obstruction". The core lies in the lungs, presenting a condition that combines internal and external factors – involving both intrinsic deficiencies and external pathogenic influences. The lungs play a pivotal role in regulating qi, and it can be said that all functions related to qi are connected to the lungs. The dispersing and descending actions of lung qi effectively propel blood circulation throughout the body. However, prolonged illness damages lung qi, leading to obstruction that impairs pulmonary circulation. This manifests as chest tightness, bloating, and restricted contraction and descent functions. Weakened lung function may further affect the kidneys, exacerbating dyspnoea characterised by shallow, laboured breathing, difficulty sustaining inhalation, and worsening symptoms during exertion. During stable phases, deficiency patterns typically stem from insufficiency in the "triple organ system of lung, spleen, and kidney," whilst excess patterns often arise from phlegm and stasis obstructions [8]. Fundamental deficiency constitutes the primary pathogenesis, while phlegm and stasis accelerate disease progression. Addressing the intertwined deficiency of lung, spleen, and kidney alongside phlegm-stasis accumulation, the hospital introduced Lung-Tonifying Granules, drawing inspiration from the Lung-Tonifying Decoction in Essential Prescriptions for Emergencies and the Golden-Water Six-Gentlemen Decoction from New Formulas of the Eight Arrays in the Complete Works of Jingyue. This formulation is employed for treating chronic obstructive pulmonary disease (COPD). Research findings indicate that Lung-Tonifying Granules significantly ameliorate patient symptoms, reduce activity limitations and disease impact, enhance quality of life, and delay pulmonary function decline, establishing it as a crucial therapeutic approach for COPD. Animal studies demonstrate that while elevating IFN- $\gamma$  levels, the Lung-Tonifying Granules markedly reduce IL-8, IL-4, and TNF- $\alpha$  concentrations in both alveolar lavage fluid and serum of asthmatic guinea pigs, thereby alleviating inflammation. Based on clinical experience, appropriate modifications to the Lung-Tonifying Granules have yielded the Lung-Tonifying and Blood-Activating Formula. This formula comprises the following Chinese medicinal herbs: Ephedra, Apricot Kernel, Codonopsis, Atractylodes, Poria, Chenpi (Tangerine Peel), Banxia (Pinellia Rhizome), Shengdihuang (Raw Rehmannia Root), Shudihuang (Processed Rehmannia Root), Shanzhuyu (Cornus Fruit), Chishao (Red Peony Root), Danggui (Angelica Root), Sangbaipi (Mulberry Bark), and Huangqin (Scutellaria Root). Taizishen fortifies the spleen and boosts qi, enhancing pulmonary function; Shudihuang and Shanzhuyu nourish kidney yin, harmonising the metal-water relationship; Danggui and Chishao replenish and activate blood circulation; Ma Huang and Xingren promote lung function and alleviate asthma. Scutellaria baicalensis possesses heat-clearing and blood-cooling properties, while Pinellia ternata and Citrus

reticulata peel facilitate qi circulation and phlegm resolution. These serve as auxiliary herbs. Collectively, the formula achieves effects of lung tonification, spleen strengthening, kidney nourishment, phlegm resolution, blood activation, and asthma alleviation. This restores qi, eliminates phlegm-dampness, and dissipates heat symptoms. Concurrently, it elevates clear qi and reduces turbid qi, harmonising spleen and lung qi to restore normal organ function.

Pulmonary diseases exhibit diverse aetiologies, complex pathogenesis, and varied pathological manifestations, with overall prevalence rates continuing to rise. Current key theories regarding this condition encompass autophagy mechanisms, airway injury, inflammatory immune responses, and oxidative stress. These theories propose that inhalation of harmful particles or gases activates pulmonary immune responses, triggering inflammatory cells to release numerous substances that cause structural lung damage. Chronic bronchitis and emphysema are common manifestations of this disease. At advanced stages, patients may develop hypoxic respiratory failure. Additionally, the disease may manifest extrapulmonary symptoms, including weight loss, systemic inflammation, malnutrition, cardiovascular complications, cancer, frailty, sarcopenia, and osteoporosis. Current Western medical treatments primarily comprise inhaled bronchodilators (such as tiotropium bromide and formoterol), combination bronchodilator-corticosteroid medications (e.g., budesonide/formoterol), acetylcysteine, and ambroxol. These drugs demonstrate significant efficacy in reducing pulmonary inflammation but may occasionally cause adverse reactions such as gastrointestinal discomfort or tachycardia<sup>[9]</sup>. Consequently, developing a universal and stable treatment approach alongside formulating individualised medication strategies has become a critical imperative in contemporary medical research.

Formulas that nourish the lungs and invigorate blood circulation can address COPD through multiple targets, including *Salvia tridentata*, dimethoxyflavonoids, *Evodia* leaf extract B, (+)-1(R)-Aconitine, and *Scutellaria baicalensis* flavonoids. Several active compounds play pivotal roles, with *Salvia tridentata* being a flavonoid recognised for its anti-inflammatory, antioxidant, and anti-tumour effects. Experiments by RAFATIAN et al. <sup>[10]</sup> demonstrate that triterpenoid saponins can inhibit apoptosis while exhibiting antioxidant properties. Furthermore, in mouse studies <sup>[11]</sup>, triterpenoid saponins demonstrated efficacy in suppressing mammary tumour proliferation, with their anti-tumour effects encompassing resistance against mammary tumours, mammary adenocarcinomas, mammary gland disorders, breast-related issues, and lactation-associated complications. Luteolin aids vasodilation, thereby enhancing blood pressure regulation <sup>[12]</sup>. Chen Yang et al. <sup>[13]</sup> discovered that triterpenoid saponins enhance  $\gamma\gamma T$  cell cytotoxicity against SW-620 colon cancer cells while stimulating  $\gamma\gamma T$  cell proliferation. This mechanism is hypothesised to involve increased PFP and GraB expression alongside ERK signalling pathway activation. Rafatian et al. <sup>[10]</sup> demonstrated that SH-SY5Y cells underwent apoptosis and autophagy under oxidative stress. *Salvia tridentata* extract, through independent antioxidant mechanisms, augmented apoptotic and autophagic effects by modulating apoptosis-related enzymatic reactions. Flavonoids are renowned for their antitumour, antiviral, antibacterial, and anti-inflammatory properties <sup>[14-17]</sup>. Flavonoids from *Andrographis paniculata* demonstrated superior free radical scavenging activity compared to its lactones, with efficacy differences ranging from 12% to 30%. These findings indicate that flavonoids in *Andrographis* exhibit more pronounced overall antioxidant performance alongside potent free radical scavenging capacity <sup>[18]</sup>. Most flavonoids exert anti-inflammatory effects through mechanisms including limiting the release of substances such as histamine from basophils and mast cells, reducing hyaluronidase activity, and diminishing neutrophil lysosomal and degranulation responses<sup>[19]</sup>. Research indicates flavonoids interact with macrophages, reducing their inflammatory state to lower inflammation risk. By regulating low-density lipoprotein cholesterol levels, flavonoids decrease blood lipids, stabilise atherosclerotic plaques, reduce plaque rupture likelihood, and slow atherosclerosis progression. They also inhibit the enhanced expression of

MMP-2 and MMP-9 in mouse peritoneal macrophages induced by oxidised LDL [20]. Modern pharmacological research indicates that *Paeonia rubra* possesses multiple efficacies, including antiplatelet aggregation, antithrombotic, anti-atherosclerotic, myocardial cell protection, microcirculation improvement, anti-inflammatory, immunomodulatory, anticonvulsant, antidepressant, and antitumour effects. These effects derive from active constituents extracted from *Paeonia lactiflora*, notably wuyao alkaloid, which exhibits significant cardiac function alongside antitumour, bronchial asthma-relieving, and anti-inflammatory properties [21]. Key active constituents include 1-spirost-9,13-diene-3,20-dione (1-SPD), stigmasterol, estrone, and licochalcone B (eB), as identified by Yang et al. [22]. Network pharmacology studies reveal that 70 target genes associated with chronic childhood cough correspond to active components in bitter almond-rapeseed extract, with machilin and other constituents equally significant. This formulation may intervene in chronic cough patients via a multi-target, multi-pathway approach. Su-chi flavonoids, belonging to the flavonoid class, exhibit anti-tumour, antiviral, and anti-inflammatory properties, offering protective effects. Chen et al. [23] demonstrated that su-chi flavonoids bind to nine specific targets. By modulating ADORA1, ADORA2A, PTGS2, CTSG, NOS2, and NOS3, it effectively reduces inflammatory responses. Through influencing the expression of PROC, SERPINE1, and F9, it inhibits coagulation processes, thereby mitigating inflammation and coagulation issues induced by sepsis, achieving the dual objectives of prevention and treatment.

PPI network analysis indicates that the primary targets of the Lung-Nourishing and Blood-Activating Formula include IL-6, IL-1 $\beta$ , STAT3, JUN, and HIF-1 $\alpha$ . Molecular docking data reveal strong binding affinity between these key active components and their targets, demonstrating high activity and favourable resistance properties. During inflammatory responses such as infection, IL-6—a multifunctional cytokine—significantly increases. It binds to GP130 on cell membranes, activating the phosphorylation of Signal Transduction and Transcription Activator 3 (STAT3) and participating in infection-related pathological processes [24]. Wu Juan et al. [25] noted that IL-6 serves as a marker of systemic inflammation, aiding in predicting disease progression in COPD patients. By promoting C-XC chemokines it facilitates neutrophil activation and aggregation in the airways, elevating the NLR ratio. This mechanism likely explains why the combined use of NLR and IL-6 enhances diagnostic accuracy [26]. From the chronic stage to acute exacerbations in COPD, NLR and IL-6 play pivotal roles in disease progression. JUN, as a key transcription factor, influences multiple cellular processes including proliferation, differentiation, migration, and apoptosis [27]. It activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway, nuclear factor- $\kappa$ B (NF- $\kappa$ B), enhances pro-inflammatory factors, and disrupts extracellular matrix (ECM) balance [28]. It affects multiple inflammatory signalling pathways, affecting pathways such as IL-1 $\beta$ , PI3K, and NF- $\kappa$ B. IL-1 $\beta$  activates the PI3K/AKT/NF- $\kappa$ B signalling cascade, implicated in various inflammation-related diseases. Research findings [29] demonstrate that IL-1 $\beta$  activates PI3K and promotes AKT phosphorylation. Inhibition of the PI3K/AKT pathway facilitates NF- $\kappa$ B phosphorylation. Activation of the NF- $\kappa$ B pathway releases multiple inflammatory factors involved in the inflammatory response, while simultaneously releasing additional inflammatory factors that further stimulate pathway activation. IL-1 $\beta$  induces macrophage secretion of matrix metalloproteinase-9, which degrades extracellular matrix components in lung tissue. This leads to disruption of elastic fibres in alveolar septa, triggering airway remodelling and fibrosis that exacerbates emphysema and worsens disease progression. JNK (Jellyfish N-terminal kinase) is the most significant member of the MAPK family. This N-terminal kinase plays a central role in numerous biological processes, including cell proliferation, DNA repair, autophagy, tumour transformation, and inflammatory responses. The JNK-specific inhibitor SP600125 prevents STAT1 translocation from the cytoplasm to the nucleus, thereby impairing STAT1's regulatory capacity.

over target genes in RAW264.7 cells [30]. Another study indicated that exposure of RAW264.7 cells to T-2 toxin activates JNK1, STAT1, and STAT3. SP600125 inhibits both IL-6 mRNA expression [31] and STAT3 activation, suggesting that JNK inhibition also suppresses STAT3, thereby reducing IL-6 release. The STAT family comprises STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6. Upon activation of receptor-associated JAKs, tyrosine residues of STATs undergo phosphorylation to form dimers, subsequently translocating to the nucleus to regulate specific gene expression [32]. Research indicates that STAT1 participates in the transcription of inducible nitric oxide synthase (iNOS) genes, while STAT3 is involved in the transcription of inflammatory factor genes such as IL-6 and IL-1 $\beta$  [33]. HIF-1 $\alpha$  is produced by cells under hypoxic conditions, primarily responsible for maintaining cellular oxygen homeostasis and promoting the expression of related genes. As a key regulator of multiple inflammatory signals, it enhances mucus secretion and accelerates the progression of chronic obstructive pulmonary disease [34]. Recent research indicates that NLRP3 expression under hypoxic conditions is regulated by HIF-1 $\alpha$  [35]. Post-transcriptionally, HIF-1 $\alpha$  may undergo degradation influenced by ubiquitin-like modification proteins such as SUMO, thereby maintaining its own equilibrium. Under normoxic conditions, HIF-1 $\alpha$  degradation occurs via the ubiquitin-proteasome pathway to regulate its levels. However, in hypoxic conditions, HIF-1 $\alpha$  degradation is inhibited, leading to its accumulation and translocation to the nucleus, thereby promoting the expression of its target genes. HIF-1 $\alpha$  can be degraded through the action of ubiquitin-like modification proteins. Hypoxic environments increase HIF-1 $\alpha$ 's ubiquitination, further enhancing proline residue hydroxylation. This enhances HIF-1 $\alpha$ 's binding affinity with VHL, ultimately leading to HIF-1 $\alpha$ 's ubiquitination and degradation [36]. Conversely, deubiquitination of HIF-1 $\alpha$  can prevent its degradation under hypoxic conditions [37,38].

In summary, this study employed network pharmacology and molecular docking methods to analyse the therapeutic mechanism of the Lung-Nourishing and Blood-Activating Formula against COPD. It demonstrates the comprehensive therapeutic characteristics of traditional Chinese medicine, involving multiple components and multiple action points when confronting disease. This provides data support and directional reference for the practical application of this formula in treating COPD. However, the study itself has limitations, as it did not fully validate the primary active components and key action points. Further investigation through more in-depth experimental approaches is required to clarify the precise mechanisms by which the Lung-Nourishing and Blood-Activating Formula influences COPD.

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