

The molecular mechanism of *Ligusticum wallichii* for improving idiopathic pulmonary fibrosis

A network pharmacology and molecular docking study

Xiaozheng Wu, PhD^{id}, Wen Li, PhD, Zhenliang Luo, PhD, Yunzhi Chen, PhD*

Abstract

Background: At present, there was no evidence that any drugs other than lung transplantation can effectively treat Idiopathic Pulmonary Fibrosis (IPF). *Ligusticum wallichii*, or Chinese name Chuan xiong has been widely used in different fibrosis fields. Our aim is to use network pharmacology and molecular docking to explore the pharmacological mechanism of the Traditional Chinese medicine (TCM) *Ligusticum wallichii* to improve IPF.

Materials and methods: The main chemical components and targets of *Ligusticum wallichii* were obtained from TCMSP, Swiss Target Prediction and PharmMapper databases, and the targets were uniformly regulated in the Uniprot protein database after the combination. The main targets of IPF were obtained through GenCards, OMIM, TTD and DRUGBANK databases, and protein interaction analysis was carried out by using String to build PPI network. Metascape platform was used to analyze its involved biological processes and pathways, and Cytoscape3.8.2 software was used to construct “component-IPF target-pathway” network. And molecular docking verification was conducted through Auto Dock software.

Results: The active ingredients of *Ligusticum wallichii* were Myricanone, Wallichilide, Perlolirine, Senkyunone, Mandenol, Sitosterol and FA. The core targets for it to improve IPF were MAPK1, MAPK14, SRC, BCL2L1, MDM2, PTGS2, TGFB2, F2, MMP2, MMP9, and so on. The molecular docking verification showed that the molecular docking affinity of the core active compounds in *Ligusticum wallichii* (Myricanone, wallichilide, Perlolirine) was <0 with MAPK1, MAPK14, and SRC. Perlolirine has the strongest molecular docking ability, and its docking ability with SRC (−6.59kJ/mol) is particularly prominent. Its biological pathway to improve IPF was mainly acted on the pathways in cancer, proteoglycans in cancer, and endocrine resistance, etc.

Conclusions: This study preliminarily identified the various molecular targets and multiple pathways of *Ligusticum wallichii* to improve IPF.

Abbreviations: ADME = Absorption, Distribution, Metabolism, Excretion, AECs = Alveolar epithelial cells, ALI = Acute lung injury, ARDS = Acute respiratory distress syndrome, BCL2L1 = Bcl-2-like 1, BP = biological processes, CC = cellular components, COVID-19 = Coronavirus Disease 2019, DL = drug-likeness, DM2 = Type 2 diabetes mellitus, EMT = Epithelial-mesenchymal transition, F2 = Coagulation factor II, FA = Folic acid, FGF = Fibroblast growth factor, GO = Gene Ontology, IL-1 = Interleukin-1, IL-6 = Interleukin-6, IPF = Idiopathic Pulmonary Fibrosis, KEGG = Kyoto Encyclopedia of Genes and Genomes, LC = lung cancer, LPS = Lipopolysaccharide, LUAD = Lung adenocarcinoma, MAPK1 = Mitogen-activated protein kinase 1, MAPK14 = Mitogen-activated protein kinase 14, MAPKs = Mitogen-activated protein kinases, MDM2 = mouse double minute 2, MF = molecular functions, MMP2 = matrix metalloproteinase 2, MMP9 = matrix metalloproteinase 9, OB = oral bioavailability, OMIM = Online Mendelian Inheritance in Man, PDB = Protein Data Bank, PDGF = Platelet-derived growth factor, PF = Pulmonary fibrosis, PPI = Protein-protein interaction, PTGS2 = Prostaglandin-endoperoxide synthase 2, SRC = Serine rich coiled-coil, TCM = Traditional Chinese medicine, TCMSP = systematic pharmacology platform of TCM, TGF = Transforming growth factor, TGFB2 = Transforming growth factor beta 2, TNF- α = Tumor necrosis factor- α , TTD = Therapeutic Target Database.

Keywords: idiopathic pulmonary fibrosis, IPF, *ligusticum wallichii*, molecular mechanism, network pharmacology

Editor: N/A.

This work was supported by the Doctor start-up fund of Guizhou University of traditional Chinese Medicine in china (Grant Number: 3043-043190021).

The authors have no conflicts of interest to disclose.

Availability of data and material: Data supporting our findings are contained within the manuscript.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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How to cite this article: Wu X, Li W, Luo Z, Chen Y. The molecular mechanism of *Ligusticum wallichii* for improving idiopathic pulmonary fibrosis: A network pharmacology and molecular docking study. *Medicine* 2022;00(00). *Medicine* 2022;101:6(e28787).

Received: 27 September 2021 / Received in final form: 20 January 2022 / Accepted: 21 January 2022

<http://dx.doi.org/10.1097/MD.0000000000028787>

1. Introduction

In recent 10 years, with the development of modern genomics,^[1–3] proteomics,^[4,5] metabolomics,^[6,7] and other “omics” theory^[8,9] and with the introduction of system biology perspective and the application of bio informatics, the concept of network Pharmacology came into being.^[10,11] It revealed the mystery of the synergistic effect of multi molecular drugs on the human body^[12] based on the “disease-gene-target- drug” interaction network and it systematically and comprehensively observed the intervention and influence of drugs on the disease network through network analysis. This is the same as the theory of treating diseases from the organic overall perspective and the principle of multi-component, multi-channel, and multi-target synergy of Traditional Chinese medicine (TCM). Undoubtedly, it has bridged the gap between TCM and conventional treatment, and pointed out the direction for the modernization and internationalization of TCM.

Idiopathic pulmonary fibrosis (IPF) is a group of diffuse lung parenchymal lesions of unknown cause.^[13] The reaction and interaction of fibroblasts and Alveolar epithelial cells (AECs) is the main link in the pathogenesis of pulmonary fibrosis.^[14] Pefenidone and Nintedanil were recommended in the 2015 Guidelines, but due to factors such as large side effects and high prices, these two drugs are not widely used.^[15] In addition, the Guidelines also pointed out that there was no evidence that any drugs other than lung transplantation can effectively treat IPF.^[16] Therefore, it is imperative to explore new drugs to control and treat it.

IPF has been recorded in ancient Chinese medical literatures, and most of them believe that it's part of the categories of “pulmonary flaccidity” and “pulmonary arthralgia.”^[17] In terms of pathogenesis, its essence is Qi deficiency and blood stasis, “ in which Qi deficiency refers to the deficiency of the lung and kidney, and blood stasis refers to the mutual obstruction of phlegm and blood stasis. Blood stasis is the most important target for the occurrence and development of IPF. It can be considered that the pathological process of IPF has blood stasis. Therefore, we should promote blood circulation and remove blood stasis in controlling and treating IPF.^[17] Ligusticum wallichii, or Chinese name Chuan xiong, has the effect of promoting blood circulation and removing blood stasis.^[18] Modern studies have found that it mainly contains volatile oils, alkaloids, organic acids and other chemical components, and it has the pharmacological effects on the treatment of hypertension, anti-thrombotic, antioxidant, neuroprotection, anti-tumor, etc,^[19–21] which can be clinically used to treat cardiovascular diseases, vertigo, kidney diseases, and so on.^[22,23] In recent years, it is also been widely used in different fibrosis fields.^[24–26] Our previous study found^[27] that the main active ingredients of Ligusticum wallichii could improve the clinical symptoms of IPF patients with good safety, and speculated that Ligusticum wallichii might “disrupt/inhibit” IPF-related target network and have a certain therapeutic effect on IPF, but its composition, target and mechanism of action are remained unclear.

This study is based on network pharmacology and molecular docking to seek the target and possible mechanism of action of Ligusticum wallichii to improve IPF, and provided a certain scientific basis for the research and clinical application of it to improve IPF.

2. Material and methods

2.1. Screening of the related targets of Ligusticum wallichii

The chemical components of Ligusticum wallichii were searched through the systematic pharmacology platform of TCM

(TCMSP),^[28] and the active components were initially screened according to the two absorption, distribution, metabolism, Excretion (ADME) attribute values with oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 to obtain the active compounds and their protein targets. In addition, the 2D structure of these active compounds was entered in Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>),^[29] and they were submitted and filtered in the database at the same time to obtain the predicted target of the molecule. The higher the probability value, the greater the possibility. Pharmmapper database (<http://www.lilab-ecust.cn/pharmmapper/>)^[30] can reversely predict potential targets and score them, where a higher Z'-score indicates a higher probability. The 3D structure of the molecule was entered and submitted in the database, and the predicted target of the molecule was selected. At the same time, known targets of unpredicted active compounds based on published literature reports were added. The targets of the three drug databases were combined after screening. In order to standardize the information of protein targets, the protein targets of compounds action were unified in the Uniprot Protein Database (<https://www.uniprot.org/>).

2.2. Screening of IPF-related targets

The potential targets for IPF treatment in the GeneCards database (<https://www.genecards.org/>), Online Mendelian Inheritance in Man (OMIM) database (<https://omim.org/>), and Therapeutic Target Database (TTD) database (<http://db.idr.blab.net/ttd/>) were explored with “Idiopathic pulmonary fibrosis” as the key word, and the DRUGBANK database (<https://go.drugbank.com/>) was searched for clinical first-line western medicine targets for IPF as supplemented.^[31] In the Genecards database, a higher Score indicates that the target is closely related to the disease. According to previous literature records, if there are too many targets, the target with a Score greater than the median is set as the potential target of IPF. Therefore, we deleted the duplicate values and finally got the target of IPF after merging the targets of the 4 disease databases.

2.3. Construction of Ligusticum wallichii-IPF target PPI Network

Jvonn online platform (<http://www.bioinformatics.com.cn/static/others/jvonn/index.html>)^[32] was used to take the intersection of the two targets and to draw the Venny diagram in order to clarify the interaction between Ligusticum wallichii related targets and IPF targets. The intersection target was submitted to STRING11.0 (<https://string-db.org/cgi/>) database to construct a Protein protein interaction (PPI) network model,^[33] and the biological species was set as “Homo sapiens.” The minimum interaction threshold was set as “medium confidence” (>0.4), and the rest settings were default settings, and the PPI network was obtained. The potential protein functional module was obtained by a further analysis of the PPI network through Metascape platform (<http://metascape.org/>), and its function was described by analyzing the biological process in which it participated.

2.4. Enrichment analysis of target functions and pathways of Ligusticum wallichii-IPF

Metascape has a comprehensive comment function and it updates the gene annotation data^[34,35] on a monthly basis. The target of

Ligusticum wallichii to improve IPF was inputted into the platform, after which we set $P < .01$ to analyze the main biological processes and metabolic pathways, and the enrichment analysis was conducted. The obtained data results were preserved and they were visualized by using the EhBIO's online platform (<http://www.ehbio.com/ImageGP/>).

2.5. Construction of Ligusticum wallichii-IPF target-signal pathway network diagram

CytoScape3.8.2 was used to construct the Ligusticum wallichii-IPF target-signal pathway network diagram. CytoScape3.8.2 built-in tools were used to analyze the effective components and the network topology parameters of the target, including Degree of connection, Betweenness and Closeness, etc, and the core target and the main active components that play a drug effect were determined according to the network topology parameters.

2.6. Molecular docking verification

The top 3 targets with Degree values in the target network diagram of Ligusticum wallichii-IPF were analyzed, and their Protein Data Bank (PDB) ID were found. The 3 targets were connected with the main active components of ligusticum wallichii. PDB format files of the 3D structure of the 3 target proteins from RSCB PDB database (<https://www.rcsb.org/>) were download; SDF format file of 2D structure of the key compounds were downloaded and selected from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), and the Open Babel GUI software

was used to convert them into mol2 format file. Auto Dock software was used to dehydrate and hydrogenate target proteins, and to convert key drug compounds and target proteins into PDBQT format. Finally, the Auto Dock program was run for molecular docking. A binding energy of less than 0 indicates that the ligand can spontaneously bind to the receptor.

3. Results

3.1. Target acquisition of active ingredients of Ligusticum wallichii

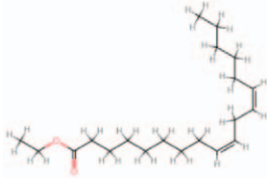
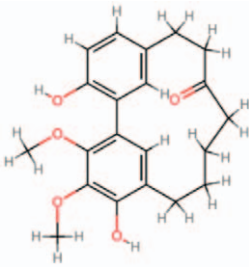
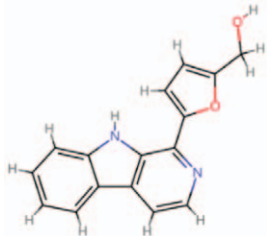
One hundred eighty-nine chemical components of Ligusticum wallichii were preliminarily extracted, and 7 active components were obtained after ADME screening, including Mandenol, Myricanone, Perlolyrine, Senkyunone, etc, as shown in Table 1. There were 262 action targets of Ligusticum wallichii component, and 185 targets were obtained by deleting duplicate values after combination.

3.2. Acquisition of IPF-related targets

Three thousand fifty-four IPF targets were obtained from Genecards database. According to previous literature,^[36] the target with score greater than the median was set as the potential target of IPF. For example, the maximum score of IPF target obtained by Genecards was 145.77, the minimum score was 0.18, and the median was 4.56, therefore, the target with score > 4.56 was set as the potential target of IPF. Related targets in database

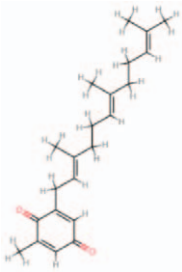
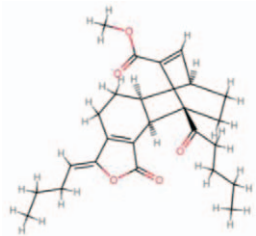
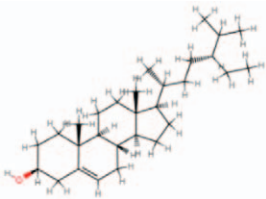
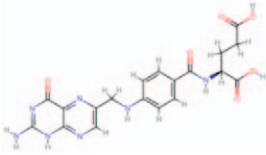
Table 1

Main components of Ligusticum wallichii.

MOL ID	Molecule name	OB (%)	DL	Molecular formula	Chemical 2D structure
MOL001494	Mandenol	42	0.19	$C_{20}H_{36}O_2$	
MOL002135	Myricanone	40.6	0.51	$C_{21}H_{24}O_5$	
MOL002140	Perlolyrine	65.95	0.27	$C_{16}H_{12}N_2O_2$	

(continued)

Table 1
(continued).

MOL ID	Molecule name	OB (%)	DL	Molecular formula	Chemical 2D structure
MOL002151	Senkyunone	47.66	0.24	C ₂₂ H ₃₀ O ₂	
MOL002157	Wallichilide	42.31	0.71	C ₂₅ H ₃₂ O ₅	
MOL000359	Sitosterol	36.91	0.75	C ₂₉ H ₅₀ O	
MOL000433	FA	68.96	0.71	C ₁₉ H ₁₉ N ₇ O ₆	

DL = drug-likeness, OB = oral bioavailability.

OMIM, TTD, and DRUGBANK were supplemented, and the duplicates were deleted after the combination, 1851 IPF-related targets were obtained.

3.3. PPI network construction of *Ligusticum wallichii*-IPF targets

The intersection of the selected active ingredient targets of *Ligusticum wallichii* and IPF disease targets was taken, and a Venny diagram was drawn through the Jvonn online platform to obtain 76 common targets of *Ligusticum wallichii* ingredient-IPF, as shown in Figure 1. Targets were then submitted to STRING11.0 platform to obtain the PPI network of *Ligusticum wallichii* target, as shown in Figure 2.

The interactions of proteins in the PPI network are usually classified as undirected graphs since they are reciprocal. PPI complex network has some regions with high density, which are called community or module. The network inside the module is the potential subnet of PPI network. The subnet connection density is high, but the regional part has few connections, so the module is considered as a collection with biological significance. This collection has two meanings: one is protein complex, that is,

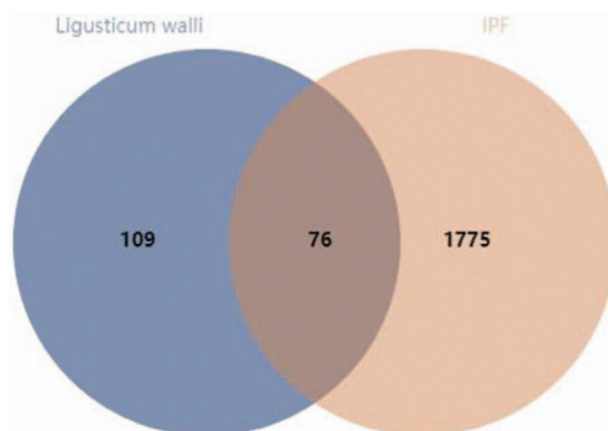


Figure 1. Venny diagram of the target of *Ligusticum wallichii* -Idiopathic Pulmonary Fibrosis (IPF): There are 185 targets for the effective ingredients of *Ligusticum wallichii*, 1851 related targets for IPF, and 76 targets for the intersection of the effective components of *Ligusticum wallichii* and IPF. (jvonn online tool, Designed by GenoToul Bioinfo and Sigenae teams).

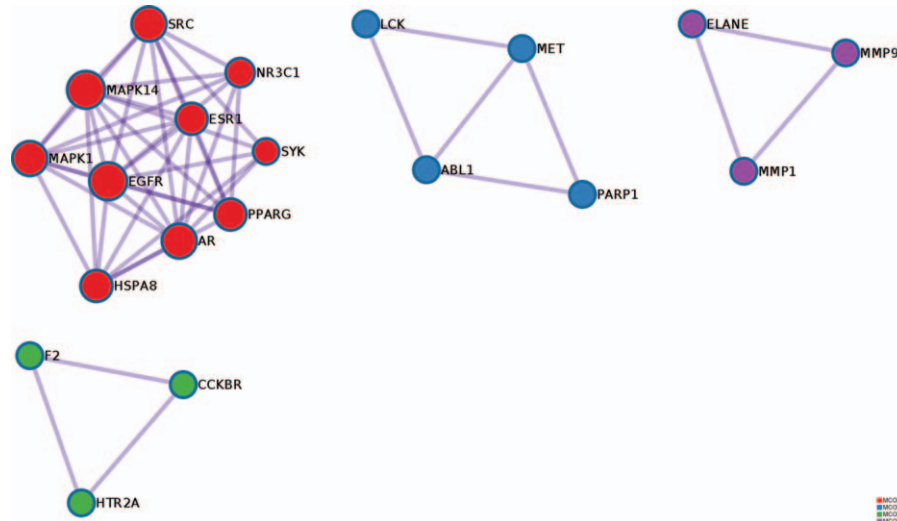


Figure 3. Module in Protein protein interaction (PPI) network of *Ligusticum wallichii*-Idiopathic Pulmonary Fibrosis (IPF) targets: The modules obtained by analyzing the interaction relationship through the molecular complex detection algorithm are mainly concentrated between Mitogen-activated protein kinase 1 (MAPK1), Mitogen-activated protein kinase 14 (MAPK14), and Serine rich coiled-coil (SRC). (Metascape data platform, Supported by National Institutes of Health (NIH) grants U19 AI106754; U19 AI135972; R01 DA03373).

ligand-receptor interaction, etc, as shown in Figures 4D and 5. The enrichment results of the target pathways are shown in Table 3.

The functions of related targets for it to improve IPF are mainly enriched in lipid binding, protein domain specific binding, cofactor binding, phosphatase binding, endopeptidase activity, and hormone binding, etc. The results are shown in Figure 4B. The cellular components involved mainly include membrane raft, ficolin-1-rich granule lumen, perinuclear region of cytoplasm, side of membrane, and postsynapse, etc. The results are shown in Figure 4C.

3.5. Construction of *Ligusticum wallichii*-IPF target-pathway network diagram

CytoScape3.8.2 was used to construct the *Ligusticum wallichii*-IPF target-pathway network diagram, as shown in Figure 6. The core components and core action targets were obtained by analyzing IPF network topology parameters of *Ligusticum wallichii* to improve IPF with built-in Network Analyzer of CytoScape3.8.2.

Cytoscape network analysis shows that Myricanone's Degree is 23, Betweenness Centrality is 0.2025, and Closeness Centrality is 0.4731, which predicts that Myricanone is the main component of *Ligusticum wallichii* to improve IPF, followed by Wallichilide (Degree is 18, Betweenness Centrality is 0.1611, and Closeness Centrality is 0.4365), and Perlolirine (Degree is 12, Betweenness Centrality is 0.0486 and Closeness Centrality is 0.4010), as

shown in Table 4. The Degree of Mitogen-activated protein kinase 1 (MAPK1) is 12, Betweenness Centrality is 0.0593, the Closeness Centrality is 0.4180, and the Degree of Mitogen-activated protein kinase 14 (MAPK14) is 12, Betweenness Centrality is 0.0897 and the Closeness Centrality is 0.4731. Therefore, MAPK1 and MAPK14 were predicted to be the main targets for it to improve IPF. Serine rich coiled-coil (SRC), Bcl-2-like 1 (BCL2L1), Mouse double minute 2 (MDM2), Prostaglandin-endoperoxide synthase 2 (PTGS2), Transforming growth factor beta 2 (TGFB2), Coagulation factor II (F2), Matrix metalloproteinase 2 (MMP2), and Matrix metalloproteinase 9 (MMP9) were also relatively important targets, as shown in Table 5.

3.6. Molecular docking verification

The top 3 core active compounds (Myricanone Wallichilide and Perlolirine) in *Ligusticum wallichii* were molecularly docked with MAPK1, MAPK14, and SRC, and it was generally believed that the lower the energy of the conformational stability of ligand and receptor binding, the greater the possibility of action. The molecular docking results showed that the molecular docking affinity of the core active compounds in *Ligusticum wallichii* was <0 with MAPK1, MAPK14, and SRC, which indicated that the core active compounds in *Ligusticum wallichii* had good binding activity with MAPK1, MAPK14, and SRC. From the perspective of binding energy, Perlolirine has the strongest molecular docking ability, and its docking ability with SRC (-6.59 kJ/mol) is particularly prominent. The results were shown in Table 6 and Figures 7–9.

4. Discussion

This study used network pharmacology and molecular docking to predict the mechanism of action of *Ligusticum wallichii* to improve IPF. The component targets and disease targets were screened through TCMSP, SwissTargetPrediction, Pharmmapper, GeneCards, OMIM, TTD, DRUGBANK database, respectively, and it

Table 2
Function description of PPI network of *Ligusticum wallichii*-IPF targets.

GO	Description	Log10 (P)
GO:0008015	Blood circulation	-22.5
GO:0003013	Circulatory system process	-22.3
GO:0071407	Cellular response to organic Cyclic compound	-20.2

GO = Gene Ontology.

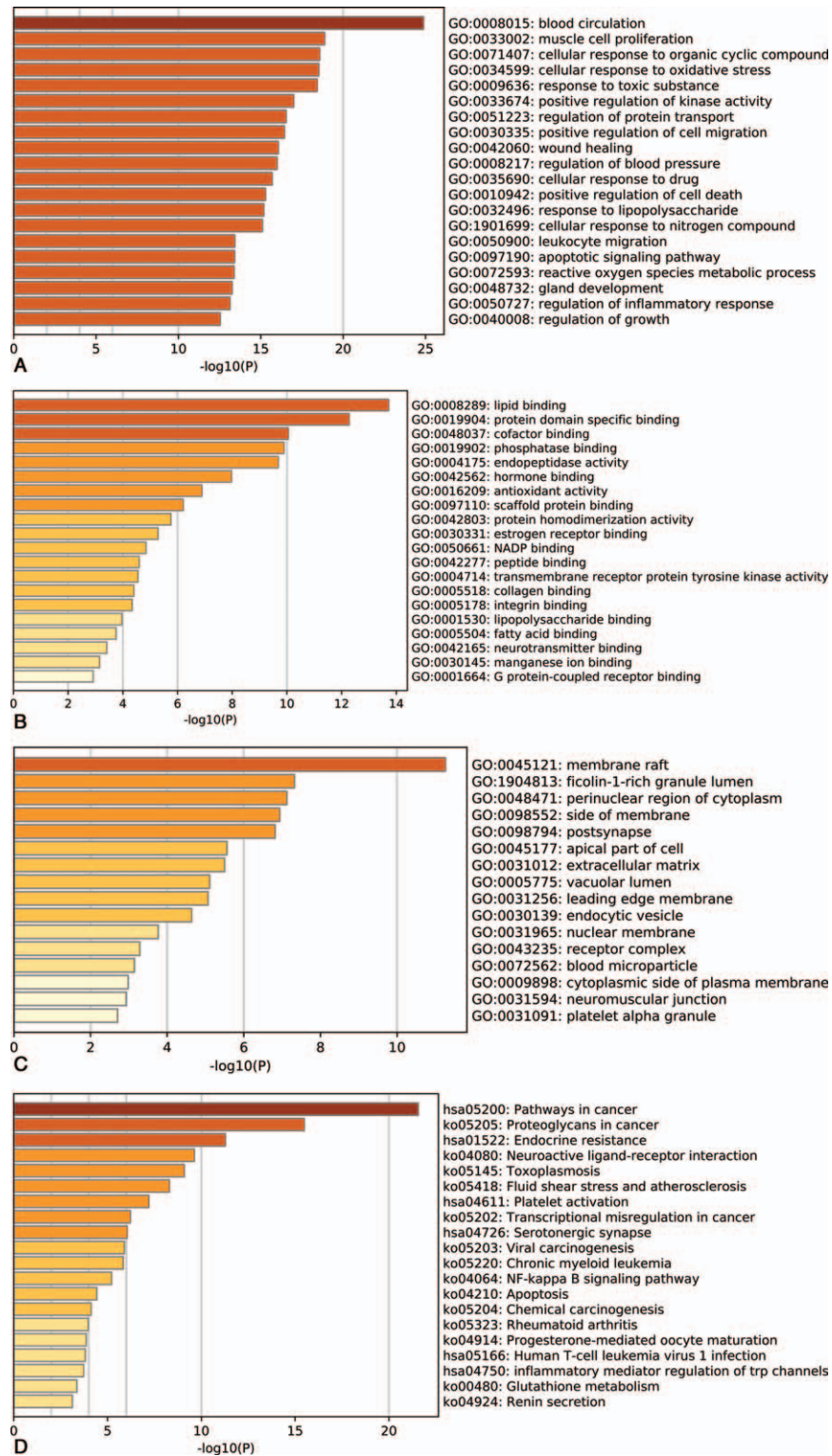


Figure 4. Enrichment analysis diagram: It is described in four parts: Biological Processes (BP) analysis, Molecular Functions (MF) analysis, Cellular Components (CC) analysis and Encyclopedia of Genes and Genomes (KEGG) analysis. (Metascape data platform, Supported by National Institutes of Health (NIH) grants U19 AI106754; U19 AI135972; R01 DA03373). (A) Gene Ontology (GO)-Biological Processes (BP) analysis: The biological processes in which *Ligusticum wallichii* are mainly involved include blood circulation, muscle cell proliferation, cellular response to organic cyclic compound, cellular response to oxidative stress, response to toxic substance, and positive regulation of kinase activity, etc. (B) Gene Ontology (GO)-Molecular Functions (MF) analysis: The functions of related targets for *Ligusticum wallichii* to improve Idiopathic Pulmonary Fibrosis (IPF) are mainly enriched in lipid binding, protein domain specific binding, cofactor binding, phosphatase binding, endopeptidase activity, and hormone binding, etc. (C) Gene Ontology (GO)-cellular components (CC) analysis: The cellular components involved mainly include membrane raft, ficolin-1-rich granule lumen, perinuclear region of cytoplasm, side of membrane, and postsynapse, etc. (D) Encyclopedia of Genes and Genomes (KEGG) analysis: The pathways involved mainly include Pathways in cancer, Proteoglycans in cancer, Endocrine resistance, and Neuroactive ligand-receptor interaction, etc.

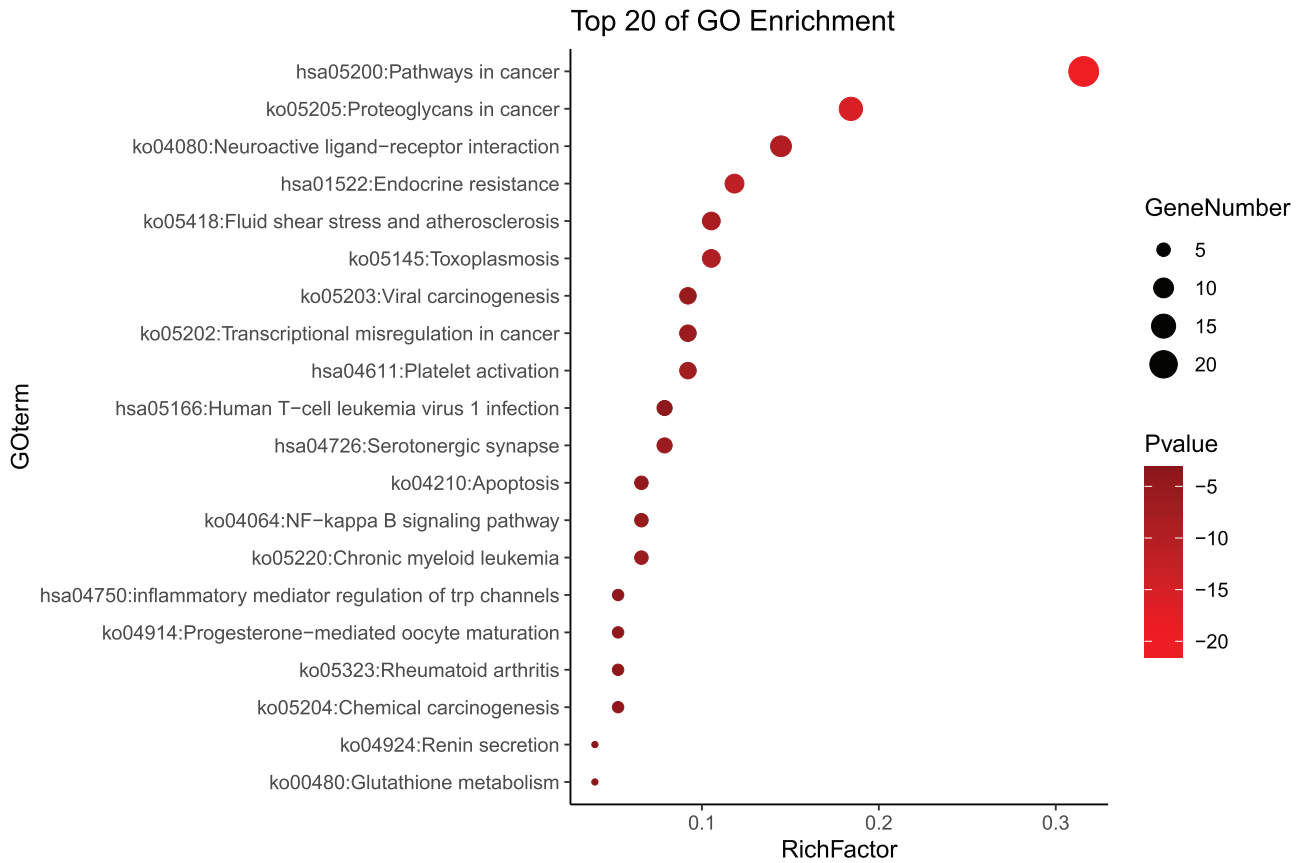


Figure 5. Bubble diagram of Encyclopedia of Genes and Genomes (KEGG) analysis: The pathways involved mainly include Pathways in cancer, Proteoglycans in cancer, Endocrine resistance, and Neuroactive ligand-receptor interaction, etc. (EhBIO's online platform, version1.0, Supported by Beijing Internet Content Provider (ICP) No. 15041106-1).

Table 3
Enrichment results of IPF target pathway in Ligusticum wallichii treatment.

GO	Description	Count	Log10 (P)	Hits
hsa05200	Pathways in cancer	24	-21.56	ABL1,AR,BCL2L1,CDC42,EGFR,ESR1,ESR2,F2,F2R,FGFR1,GSTP1,HMOX1,JAK2,JAK3,MDM2,MET,MMP1,MMP2,MMP9,NOS2,PPARG,MAPK1,PTGS2,TGFB2
ko05205	Proteoglycans in cancer	14	-15.48	CDC42,MAPK14,CTSL,EGFR,ESR1,FGFR1,KDR,MDM2,MET,MMP2,MMP9,MAPK1, SRC,TGFB2
hsa01522	Endocrine resistance	9	-11.28	MAPK14,EGFR,ESR1,ESR2,MDM2,MMP2,MMP9,MAPK1, SRC
ko04080	Neuroactive ligand-receptor interaction	11	-9.63	ADORA2A,ADRB2,CCKBR,CRHR1,DRD2,F2,F2R,NR3C1,HTR2A,P2RX7,TRPV1
ko05145	Toxoplasmosis	8	-9.08	ALOX5,BCL2L1,MAPK14,HSPA8,JAK2,NOS2,MAPK1,TGFB2
ko05418	Fluid shear stress and atherosclerosis	8	-8.3	MAPK14,CTSL,GSTP1,HMOX1,KDR,MMP2,MMP9, SRC
hsa04611	Platelet activation	7	-7.2	MAPK14,F2,F2R,MAPK1,PTGS1, SRC, SYK
ko05202	Transcriptional misregulation in cancer	7	-6.22	BCL2L1,ELANE,MDM2,MET,MMP3,MMP9,PPARG
hsa04726	Serotonergic synapse	6	-6.05	ALOX5,CYP2C9,HTR2A,MAPK1,PTGS1,PTGS2
ko05203	Viral carcinogenesis	7	-5.89	CCNA2,CDC42,JAK3,MDM2,MAPK1, SRC, SYK
ko05220	Chronic myeloid leukemia	5	-5.84	ABL1,BCL2L1,MDM2,MAPK1,TGFB2
ko04064	NF-kappa B signaling pathway	5	-5.21	PARP1,BCL2L1,LCK,PTGS2, SYK
ko04210	Apoptosis	5	-4.42	PARP1,BCL2L1,CTSB,CTSL,MAPK1
ko05204	Chemical carcinogenesis	4	-4.13	CYP2C9,EPHX1,GSTP1,PTGS2
ko05323	Rheumatoid arthritis	4	-3.97	CTSL,MMP1,MMP3,TGFB2
ko04914	Progesterone-mediated oocyte maturation	4	-3.86	CCNA2,MAPK14,PGR,MAPK1
hsa05166	Human T-cell leukemia virus 1 infection	6	-3.81	BCL2L1,CCNA2,JAK3,LCK,MAPK1,TGFB2
hsa04750	inflammatory mediator regulation of trp channels	4	-3.73	MAPK14,HTR2A, SRC, TRPV1
ko00480	Glutathione metabolism	3	-3.36	G6PD,GSR,GSTP1
ko04924	Renin secretion	3	-3.13	ADRB2,CTSB,REN

GO = Gene Ontology.

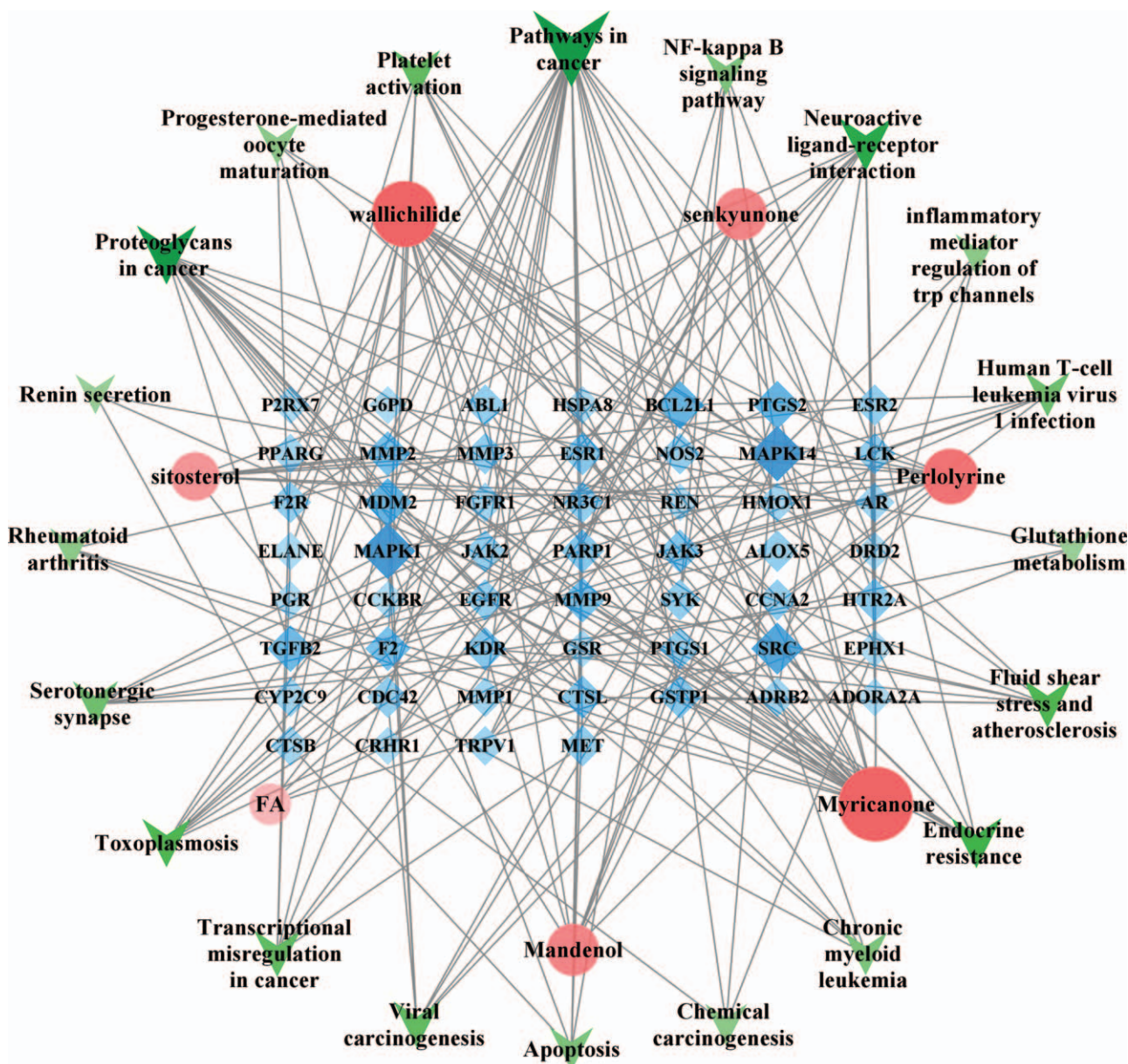


Figure 6. Ligusticum wallichii component-Idiopathic Pulmonary Fibrosis (IPF) target-pathway network diagram: Myricanone's Degree is 23, Betweenness Centrality is 0.2025, and Closeness Centrality is 0.4731, Wallichilide's Degree is 18, Betweenness Centrality is 0.1611, and Closeness Centrality is 0.4365, and Perlolyrine's Degree is 12, Betweenness Centrality is 0.0486 and Closeness Centrality is 0.4010, which predicts that Myricanone, Wallichilide and Perlolyrine are the main components of Ligusticum wallichii to improve IPF. The main targets are Mitogen-activated protein kinase 1 (MAPK1), Mitogen-activated protein kinase 14 (MAPK14), and Serine rich coiled-coil (SRC). The main signaling pathways are Pathways in cancer, Proteoglycans in cancer, Endocrine resistance and Neuroactive ligand-receptor interaction. (Cytoscape, version3.8.2, provided by the U.S. National Institute of General Medical Sciences (NIGMS) R01 GM070743).

was found that there were 76 intersection target proteins related to IPF in Ligusticum wallichii. PPI interaction analysis and the construction of Ligusticum wallichii—IPF targets—pathway network was conducted afterwards, and the core components (Myricanone, Wallichilide, Perlolyrine, etc) and the main action targets (MAPK1, MAPK14, SRC, BCL2L1, MDM2, PTGS2, TGFB2, F2, MMP2, and MMP9) were obtained.

Myricanone is a cyclodiaryl heptadecane macrocyclic compound with strong anti-cancer activity.^[38] It has been reported^[39,40] that myricetone has the effects of regulating lipid metabolism, anti-oxidation, improving insulin resistance, and antihyperglycemic. Like Ligustrazine, Perlolyrine is an alkaloid

nitrogenous compound. Experimental studies have shown that it has a good anti-myocardial ischemia effect.^[41]

In addition, Perlolyrine and its analogues have the function of anticoagulant, reducing blood viscosity and erythrocyte aggregation, improving Hemorheology and slightly enhancing erythrocyte deformation.^[42] The results of molecular docking verification in this study also showed that Myricanone, Wallichilide and Perlolyrine had strong binding activity with the main targets (MAPK1, MAPK14, and SRC). Therefore, Ligusticum wallichii may mainly improve IPF with Myricanone, Wallichilide, and Perlolyrine. Among them, Perlolyrine and SRC have the most obvious effects.

Table 4**Characteristic parameters of network nodes of main active components of *Ligusticum wallichii*.**

MOLID	Name	Degree	Betweenness centrality	Closeness centrality
MOL002135	Myricanone	23	0.202482297	0.473053892
MOL002157	Wallichilide	18	0.16105415	0.436464088
MOL002140	Perlolyrine	12	0.048639321	0.401015228
MOL001494	Mandenol	10	0.042698916	0.370892019
MOL002151	Senkyunone	10	0.059994838	0.374407583
MOL000359	Sitosterol	8	0.053058758	0.314741036
MOL000433	FA	4	0.021555229	0.330543933

FA=folic acid.

Table 5**Characteristic parameters of target nodes of main active components of *Ligusticum wallichii*.**

Targets	Degree	Betweenness centrality	Closeness centrality
MAPK1	12	0.059318395	0.417989418
MAPK14	12	0.089716981	0.473053892
SRC	10	0.05939737	0.441340782
BCL2L1	8	0.034413405	0.413612565
MDM2	8	0.031052031	0.401015228
PTGS2	8	0.049435086	0.431693989
TGFB2	7	0.01874065	0.370892019
F2	6	0.035689262	0.413612565
MMP2	6	0.021793425	0.389162562
MMP9	6	0.010074407	0.381642512

BCL2L1=Bcl-2-like 1, F2=coagulation factor II, MAPK1=mitogen-activated protein kinase 1, MAPK14=mitogen-activated protein kinase 14, MDM2=mouse double minute 2, MMP2=matrix metalloproteinase 2, MMP9=matrix metalloproteinase 9, PTGS2=prostaglandin-endoperoxide synthase 2, SRC=serine rich coiled-coil, TGFB2=transforming growth factor beta 2.

The results of this study showed that the targets of *Ligusticum wallichii* to improve of IPF were mainly concentrated in MAPK1 and MAPK14, and SRC was also one of the important targets. MAPK1 and MAPK14 are members of the Mitogen-activated protein kinases (MAPKs) family, which have the functions of regulating oxidative stress response, anti-inflammatory, regulating immune response, controlling cell apoptosis and cell proliferation.^[43] The MAPK1 signaling pathway can promote the production of a variety of inflammatory factors by macrophages, such as Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), etc, which are involved in the inflammatory response of Acute lung injury/Acute respiratory distress syndrome (ALI/ARDS),^[44,45,46,47] but the influence of various inflammatory diseases can be eliminated by inhibiting the signaling pathway of MAPK1.^[48] Studies have shown that the

effect of eliminating inflammation can be achieved by silencing MAPK1 in Lipopolysaccharide (LPS)-induced A549 cells.^[49] Furthermore, MAPK1 gene knockout can inhibit the proliferation and migration in Lung Adenocarcinoma (LUAD) cells.^[50] MAPK14 is the same as MAPK1, silencing MAPK14 can also inhibit the expression of related inflammatory cytokines and promote cell proliferation and inhibit cell apoptosis in lung tissue, so as to achieve the purpose of treating ALI.^[51] SRC family kinases can be activated by cytokines that can promote the fibrosis, such as Transforming growth factor (TGF) and Platelet-derived growth factor (PDGF), and participate in the occurrence and development of Pulmonary fibrosis (PF).^[52,53] It can also regulate the migration of fibroblasts. Studies have found that extracellular matrix proteins are significantly reduced when SRC inhibitors are used, and the differentiation of myofibroblasts and the production of lung fibrosis in the lung tissue of mice are blocked.^[54] In addition, lung inflammation-related lung fibrosis and epithelial cell apoptosis induced by SRC signaling pathway can be inhibited by Nintedanib.^[55] Therefore, MAPK1, MAPK14, and SRC are closely related to the generation of inflammation, cell proliferation, inhibition of apoptosis, generation of fibrosis, and other pathological processes in lung tissues.

This study has found that *Ligusticum wallichii* improved IPF mainly through the regulation in Pathways in cancer, Proteoglycans in cancer, Endocrine resistance, Neuroactive ligand-receptor interaction and other pathways by using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Current studies believe that despite the different cell origins and pathological phenotypes, the pathogenesis of IPF and Lung cancer (LC) are highly similar. They both have chronic damage to alveolar epithelial cells accompanied by abnormal tissue repair and damage to alveolar structure. They both have excessive proliferation and the involvement of Epithelial-mesenchymal transition (EMT) of specific cell types in the lung, and there are

Table 6**Binding energies of molecular docking of core compounds in *Ligusticum wallichii*.**

MOLID	Compound	Molecular formula	Molecular weight (g/mol)	CAS	Target protein	Binding energy (kJ/mol)
MOL002135	Myricanone	C ₂₁ H ₂₄ O ₅	356.4	32492-74-3	MAPK1	-5.64
					MAPK14	-4.51
					SRC	-4.72
MOL002157	Wallichilide	C ₂₅ H ₃₂ O ₅	412.5	93236-64-7	MAPK1	-1.65
					MAPK14	-2.33
					SRC	-4.25
MOL002140	Perlolyrine	C ₁₆ H ₁₂ N ₂ O ₂	264.28	29700-20-7	MAPK1	-5.88
					MAPK14	-5.73
					SRC	-6.59

MAPK1=mitogen-activated protein kinase 1, MAPK14=mitogen-activated protein kinase 14, SRC=serine rich coiled-coil.

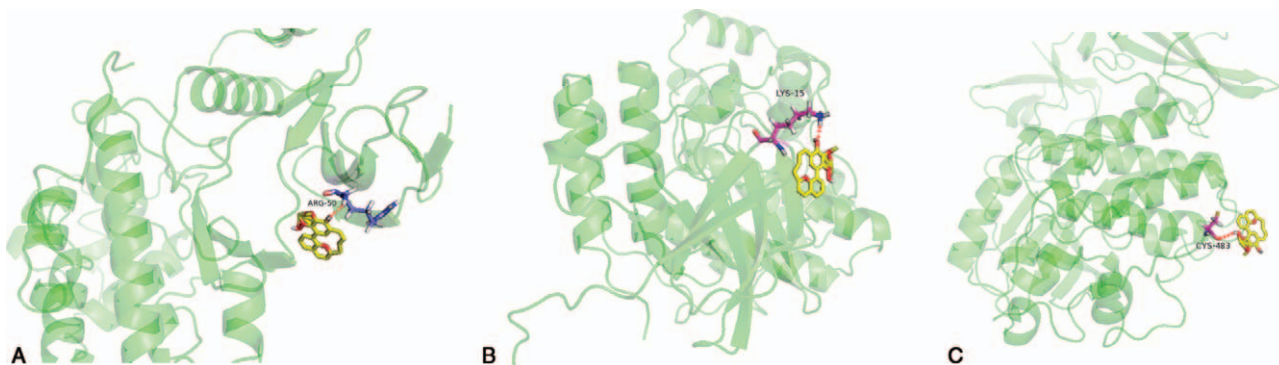


Figure 7. Molecular docking pattern diagram: It is described in three parts: Myricanone-Mitogen-activated protein kinase 1 (MAPK1), Myricanone-Mitogen-activated protein kinase 14 (MAPK14), Myricanone-Serine rich coiled-coil (SRC). (AutoDock, version4.2.6, Supported by The Scripps Research Institute). (A) Myricanone-MAPK1: They are docked at ARG-50, and the binding energy is -5.64 kJ/mol. (B) Myricanone-MAPK14: They are docked at LYS-15, and the binding energy is -4.51 kJ/mol. (C) Myricanone-SRC: They are docked at CYS-483, and the binding energy is -4.72 kJ/mol.

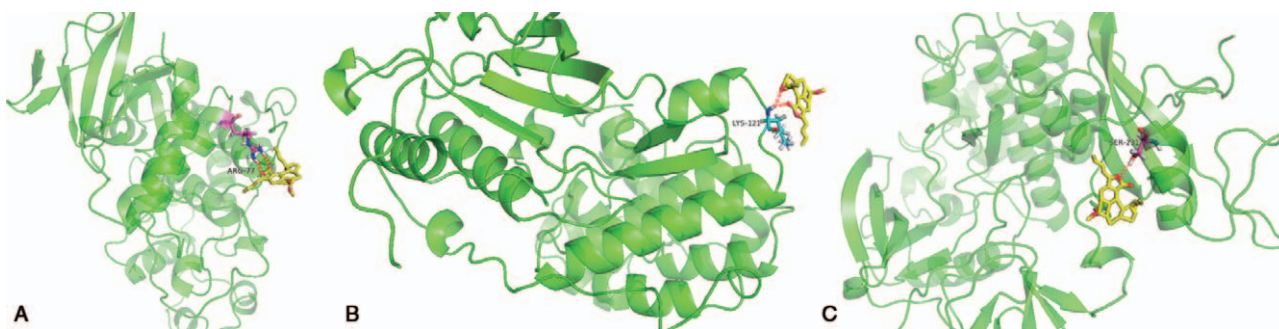


Figure 8. Molecular docking pattern diagram: It is described in three parts: Wallichilide-Mitogen-activated protein kinase 1 (MAPK1), Wallichilide-Mitogen-activated protein kinase 14 (MAPK14), Wallichilide-Serine rich coiled-coil (SRC). (AutoDock, version4.2.6, Supported by The Scripps Research Institute). (A) Wallichilide-MAPK1: They are docked at ARG-77, and the binding energy is -1.65 kJ/mol. (B) Wallichilide-MAPK14: They are docked at LYS-121, and the binding energy is -2.33 kJ/mol. (C) Wallichilide-SRC: They are docked at SER-231, and the binding energy is -4.25 kJ/mol.

similar epigenetics and activation of specific signaling pathways such as Fibroblast growth factor (FGF), etc.^[56,57] Therefore, it can be considered that the mechanism of IPF and cancer are similar, and the related cytokines can act on Pathways in cancer to induce the production IPF. In addition, there are few reports about the relationship between IPF and Endocrine resistance, but

there are more reports on the relationship with liver fibrosis. Research reports have shown that there is a risk of liver fibrosis during the pathogenesis of type 2 diabetes mellitus (DM2), and the primary cause is closely related to insulin resistance.^[58]

However, due to the limitations of network pharmacology methodology and the complexity of Chinese medicine compo-

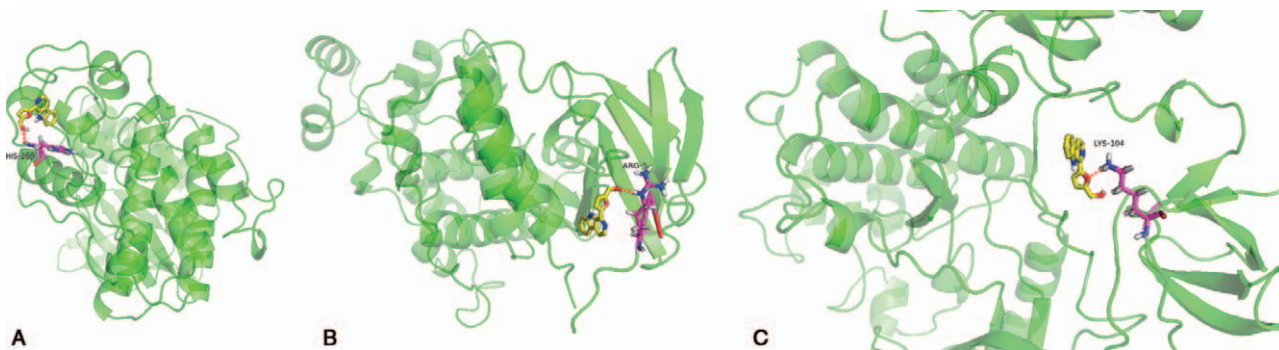


Figure 9. Molecular docking pattern diagram: It is described in three parts: Peroloryrine-Mitogen-activated protein kinase 1 (MAPK1), Peroloryrine-Mitogen-activated protein kinase 14 (MAPK14), Peroloryrine-Serine rich coiled-coil (SRC). (AutoDock, version4.2.6, Supported by The Scripps Research Institute). (A) Peroloryrine-MAPK1: They are docked at HIS-269, and the binding energy is -5.88 kJ/mol. (B) Peroloryrine-MAPK14: They are docked at ARG-5, and the binding energy is -5.73 kJ/mol. (C) Peroloryrine-SRC: They are docked at LYS-104, and the binding energy is -6.59 kJ/mol.

nents, this study could only initially explain the mechanism of *Ligusticum wallichii* to improve IPF based on the results of bioinformatics and mass data calculation combined with network pharmacology from the perspective of the organic wholeness concept of Chinese medicine. It is suggested that subsequent screening and experiments *in vivo* or *in vitro* should be conducted to verify the main regulatory targets of *Ligusticum wallichii* on the basis of the results of this study.

5. Conclusions

Ligusticum wallichii may act on relevant targets such as MAPK1, MAPK14, and SRC through its function of improving insulin resistance, improving blood fluidity, anti-cancer activity and antioxidant and through tumor-related signaling pathways and endocrine resistance pathways to play its role in anti-inflammatory, anti-oxidative stress response, suppressing immune response and cell proliferation, increasing cell apoptosis, reducing the production of extracellular matrix protein and blocking the differentiation of myofibroblasts in the lung tissue to improve IPF. Among them, perlolyrine, its main core component, has the most obvious effect on SRC, the target of IPF. This conclusion proves that the same compound of *Ligusticum wallichii* can regulate different targets, while the same target can interfere with different biological processes and signal pathways, which reflects the characteristics of the combined action of multi-pathway and multi-target of *Ligusticum wallichii*. This suggests that the entire network can be adjusted by regulating a single or multiple important targets in the network, which is also in line with the organic wholeness concept of Chinese medicine. It provides a scientific basis for the clinical application of *Ligusticum wallichii* to improve IPF and a target for the follow-up review of *Ligusticum wallichii*'s clinical efficacy evaluation indicators, and also provides a new direction for exploring the potential mechanism of *Ligusticum wallichii*.

Declaration of Figures Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Author contributions

This study is initiated by Xiaozheng Wu. Xiaozheng Wu will develop the search strategies, conduct data collection, and analyze independently.

Wen Li and Zhenliang Luo will revise it.

All authors have approved the final manuscript.

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Visualization: Xiaozheng Wu.

Writing – original draft: Xiaozheng Wu.

Writing – review & editing: Xiaozheng Wu, Zhenliang Luo.

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