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Research Article

Based on network pharmacology, the active components and molecular mechanisms of loquat leaves in the treatment of acute respiratory distress syndrome were explored

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Abstract Through the integration of network pharmacology and molecular docking methods, we analyzed and predicted the active components of loquat leaves and their molecular mechanisms in the treatment of acute respiratory distress syndrome (ARDS). The targets of the active ingredients in loquat leaves were sourced from the Swiss Target Prediction database, while the targets associated with ARDS were obtained from the GeneCards, DisGeNET, and OMIM databases. A protein-protein interaction (PPI) network was constructed using Cytoscape. Following topological analysis, core targets were identified. These core targets were further analyzed using the DAVID platform, where Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted. Molecular docking was performed using PyMOL and AutoDock Tools software, which identified 15 key active components and 17 core targets. The key pathways implicated included the HIF-1 signaling pathway, estrogen signaling pathway, microRNAs, and cancer-related pathways. The results of the molecular docking indicated that the binding affinities of the primary active components of loquat leaves, such as isorhamnetin, kaempferol, cinchonastatin 1c, and farnesiflo A, to 16 core target proteins were ≤ −6.8. Notably, cinchonain 1c exhibited the highest docking score with 8 core targets, with a particularly strong binding affinity to SRC, recorded at -12.1. The analyses conducted through network pharmacology and molecular docking suggest that loquat leaves have the potential to ameliorate acute lung injury through hypoxia-induced regulation and anti-inflammatory effects, thereby providing a clear direction for future pharmacological studies and drug development.

Keywords: network pharmacology; Loquat Leaf; acute respiratory distress syndrome; molecular mechanism

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Introduction

The loquat (Eriobotrya japonica), a subtropical evergreen fruit tree belonging to the Rosaceae family, is native to China and Japan and has been widely cultivated in Southern Europe. Its leaves are commonly used in traditional medicine after processing steps such as harvesting and sun-drying. Loquat leaves are rich in diverse chemical constituents, primarily including

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terpenoids, polyphenols, flavonoids, and various trace elements [1]. Modern pharmacological studies have demonstrated that loquat leaf extracts exhibit multiple bioactivities, such as hypoglycemic effects [2], improvement of insulin resistance [3], antitussive and antiasthmatic properties [4], antioxidant [5], antibacterial [6], antitumor [7], and metabolic regulation [8]. Consequently, these extracts are clinically applied in treating diabetes, chronic bronchitis, and malignancies. Studies indicate that loquat leaf extracts can mitigate infection-induced acute lung injury (ALI) [9].

Acute respiratory distress syndrome (ARDS) is a severe clinical syndrome characterized by acute respiratory failure, often associated with pneumonia, trauma, or severe infections. ARDS has a high incidence in intensive care units, with mortality rates reaching 30–40%, making it a critical public health concern [10]. Despite advances in supportive care, no targeted pharmacotherapy exists, and treatment remains reliant on mechanical ventilation and symptomatic management [11]. The complexity and heterogeneity of ARDS pose significant challenges in developing effective therapies.

Although numerous studies have explored ARDS pathophysiology, key knowledge gaps persist. Current literature primarily focuses on clinical features, diagnostic criteria, and supportive therapies, while mechanistic insights remain limited [12]. This underscores the urgent need for novel treatments to reduce mortality and improve outcomes. Given the traditional use of herbal medicine in respiratory diseases, investigating the therapeutic potential of loquat leaf active components in ARDS may offer breakthroughs [13].

In this context, we employed a systems biology approach, integrating databases such as TCMSP and PubChem, to screen loquat leaf's bioactive compounds and elucidate their mechanisms in ARDS. Through target prediction, protein-protein interaction (PPI) network construction, and GO/KEGG pathway analyses, we systematically deciphered the compound-target-disease relationships. This strategy provides a holistic understanding of loquat leaf's therapeutic effects, offering a theoretical foundation for novel interventions.

Objectives

This study aims to investigate the mechanisms of loquat leaf active components in ARDS treatment. By combining bioinformatics analyses, we identify ARDS-related targets to guide clinical translation. Our systematic approach not only reveals the anti-inflammatory and antioxidant properties of these components but also lays the groundwork for experimental validation and therapeutic development, ultimately improving ARDS patient outcomes.

1. Materials and Methods

1.1 Screening of Loquat Leaf Active Components

The TCMSP database (https://old.tcmsp-e.com/tcmsp.php) was queried to identify bioactive compounds using thresholds of drug-likeness (DL) > 0.18 and oral bioavailability (OB) > 30%. Candidate components were recorded for further analysis.

1.2 Target Prediction and Interaction Network

Compound structures were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and TCMSP, converted to SMILES format, and submitted to Swiss Target Prediction (http://swisstargetprediction.ch/) for human target prediction (probability cutoff: 0.1). Compound-target networks were visualized using Cytoscape 3.9.0.

1.3 ARDS-Related Target Collection

ARDS targets were retrieved from GeneCards (https://www.genecards.org/), DisGeNET (https://www.disgenet.org/), and OMIM (https://www.omim.org/) using "Acute Respiratory Distress Syndrome" as the search term. Duplicates were removed to generate a final target list.

1.4 PPI Network Construction and Core Gene Identification

Common targets of compounds and ARDS were analyzed via a Venn diagram, followed by PPI network construction using STRING and Cytoscape 3.9. Topological parameters (degree, betweenness, closeness) were calculated using the Network Analyzer plugin.

1.5 GO and KEGG Pathway Analysis

Core targets were uploaded to DAVID and Metascape for GO enrichment and KEGG pathway analysis (significance: $P < 0.01$). Species was set to Homo sapiens.

1.6 Molecular Docking

Active compounds (MOL files from TCMSP) and core protein structures (PDB IDs from RCSB, filtered by Homo sapiens and X-ray resolution) were docked using PyMOL 2.5.0 and AutoDock Tools 1.5.7 to evaluate binding affinities.

2. Results

2.1 Active Component Screening

Eighteen compounds meeting OB and DL criteria were identified from TCMSP (see Table 1).

Table 1. Information table of 18 active ingredients of Loquat leaf

Mol ID	Molecule Name	English Name	Molecular Weight (MW)	Oral Bioavailability (OB %)	Drug-likeness (DL)
MOL012556	23-trans-p-coumaryhormentic acid	23-trans-p-coumary hormentic acid	650.93	36.08	0.32
MOL012577	(2R,3R,10S)-2,10-bis(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4,9,10-tetrahydro-2H-pyrano[6,5-h]chromen-8-one	Cinchonain 1b	452.44	65.26	0.93
MOL012578	Cinchonain 1a	Cinchonain 1a	452.44	30.12	0.93
MOL012579	(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	Cinchonain 1c	452.44	58.16	0.93
MOL012581	(4S,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	Cinchonain 1d	452.44	31.32	0.93
MOL012583	(2R,3R,4S)-2-(3,4-dihydroxyphenyl)-4-(2,4,6-trihydroxyphenyl)chroman-3,5,7-triol	Prodelphinidin B6	414.39	72.41	0.64
MOL012588	7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin	Farnesiferol A	382.54	42.36	0.64

MOL012593	isohumbertiol-3-o-{α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→6)]}-β-D-glucopyranoside	Isohumbertiol-3-O-{α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→6)]}-β-D-glucopyranoside	690.87	36.75	0.59
MOL012617	Torulene	Torulene	534.94	33.49	0.55
MOL000354	isorhamnetin	Isorhamnetin	316.28	49.60	0.31
MOL000358	beta-sitosterol	β-Sitosterol	414.79	36.91	0.75
MOL000422	kaempferol	Kaempferol	286.25	41.88	0.24
MOL000098	quercetin	Quercetin	302.25	46.43	0.28
MOL000211	Mairin	Betulinic acid	456.78	55.38	0.78
MOL001002	ellagic acid	Ellagic acid	302.20	43.06	0.43
MOL001663	(4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylic acid	3-Epi-oleanolic acid	456.78	32.03	0.76
MOL006821	(-)-epigallocatechin-3-gallate	(-)-Epigallocatechin-3-gallate	458.40	55.09	0.77
MOL008173	daucosterol_qt	Daucosterol	414.79	36.91	0.75

2.3 Construction of the Drug-Active Component-Target Network

The information of active ingredients and targets collected in Section 2.2 was imported into Cytoscape 3.7.0 software, and finally the "drug - active ingredient - acting gene" network diagram was established. The specific results are referred to in Figure 1. This network graph contains 104 nodes and 291 edges. The lines connecting nodes indicate their interaction. The density of the lines represents the closeness of the interaction. The denser the lines, the closer the connection between the two. Among them, orange, blue-green and purple respectively refer to loquat leaves, active ingredients of the drug and the corresponding genes of the active ingredients. According to the analysis of the degree value, the larger the degree value, the larger the circle; conversely, the smaller the degree value, the smaller the circle.

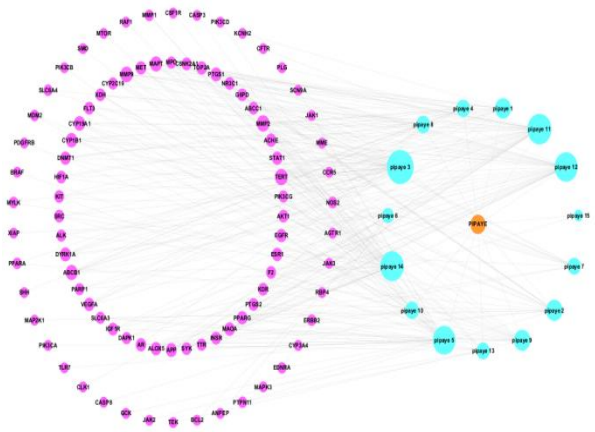


Fig. 1 Component-actiongene network of loquatleaf

2.4 Acquisition of Intersection Genes

Using Venny 2.1 software, 366 active ingredient targets and 1,910 targets of ARDS diseases were analyzed, and a Venn

diagram was drawn, as shown in Figure 2. The overlapping area is the intersection gene of the two, and a total of 88 common targets were obtained.

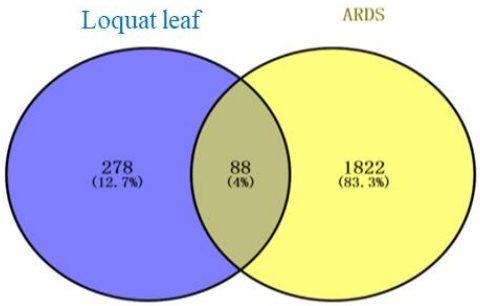


Fig. 2 Venn diagram of the targets of Loquat leaf and ARDS Syndrome

2.5 Construction and Analysis of the "Active Ingredient - Disease Target" network diagram

The 88 screened active ingredients and disease intersection targets were transferred to the STRING database, and the PPI network diagram was obtained. For details, please refer to Figure 3, and the TSV format of the file was downloaded.

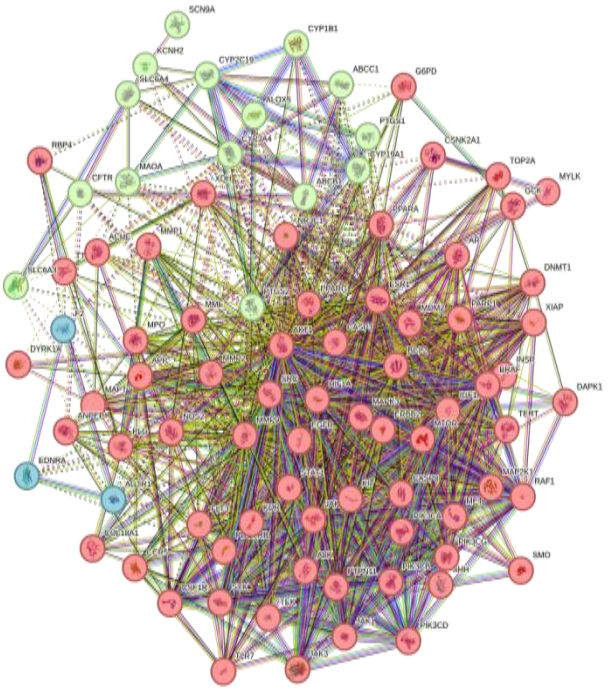


Fig. 3 The protein-protein interaction network of Loquat leaf and ARDS mellitus

2.6 Screening of Core Targets

The TSV format file obtained in 2.5 was imported into Cytoscape3.9.0 to generate the PPI graph, which has a total of 87 nodes and 1124 edges, and the potential targets of the drug's action on the disease were obtained, as shown in Figure 4A; Using the plugin Centiscape2.2, filtering was conducted with parameters such as Degree, Betweenness, and Closeness to eliminate isolated nodes and obtain the main target points, as shown in Figure 4B; Calculate the size of the degree value. The smaller the degree value, the smaller the area of the graph; conversely, the larger the degree value, the larger the area of the graph. The larger the degree value is, the greater the importance of the node in the network is. Among them, under the conditions of Closeness>0.0067388015573566, Betweenness>65.8160919540229, and Degree>25.8390804597701, 17 core targets were screened out. At this time, there were 17 nodes and 130 edges, as shown in Figure 4C. It indicates that the pharmacological effect of loquat leaves in treating acute respiratory distress syndrome mainly works through the above core targets, and its topological properties are shown in Table 2.

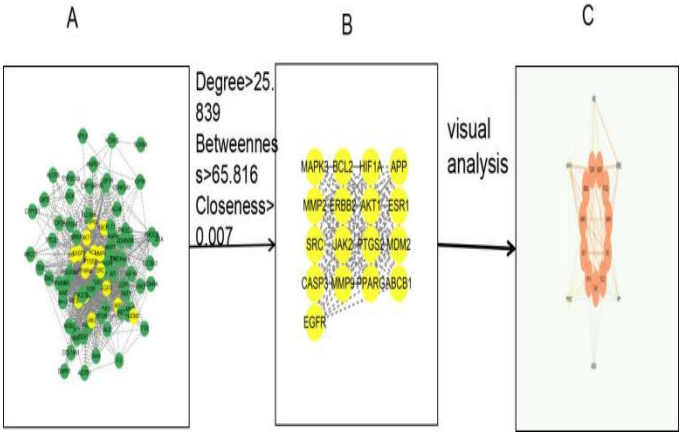


Fig. 4 Screening process of core targets.

Table 2. Key targets and topological properties of loquat leaf in the treatment of ARDS

Targets	Betweenness	Closeness	Degree
AKT1	764.0562681	0.009803922	71
EGFR	396.7888053	0.009174312	64
BCL2	304.7280024	0.009009009	62
CASP3	225.6949081	0.008695652	58
SRC	366.5872704	0.008547009	55
HIF1A	200.3015224	0.008403361	54
MMP9	164.9080543	0.008333333	53
ESR1	269.5356544	0.008333333	53
ERBB2	138.2450842	0.008196721	51
PTGS2	215.0025414	0.008130081	50
MAPK3	142.5685229	0.008064516	49
PPARG	243.6625269	0.008064516	49
JAK2	120.682688	0.007874016	46
MDM2	118.7321383	0.007575758	41
MMP2	78.85937782	0.007462687	39
ABCB1	174.7861527	0.007142857	32
APP	122.2400341	0.007042254	31

2.7 GO analysis of molecular Functions (MF), biological processes (BP), and cellular components (CC)

After calculation, 68 BP was enriched. It mainly involves the response to xenobiotic stimulus, the positive regulation of apoptotic process, and the cellular response to hypoxia response to hypoxia, positive regulation of vascular smooth muscle cell proliferation, and cellular response to reactive oxygen species response to reactive oxygen species, etc. There are a total of 15 CCS, mainly including macromolecular complexes, cytoplasm, caveola, nucleus, membrane raft, etc. 18 MFS It mainly involves enzyme binding, identical protein binding and nitric oxide synthase regulator activity activity, protein tyrosine kinase activity, etc. The result is shown in Figure 5.

response to xenobiotic stimulus	cellular response to hypoxia
cellular response to reactive oxygen species	positive regulation of vascular smooth muscle cellproliferation
positive regulation of apoptotic process	positive regulation of peptidyl-serine phosphorylation
negative regulation of intrinsic apoptotic signaling pathway	negative regulation of apoptotic process
cellular response to cadmium ion	negative regulation of gene expression
macromolecular complex	cytoplasm
caveola	nucleus
membrane raft	receptor complex
cytosol	euchromatin
nucleoplasm	perinuclear region of cytoplasm
enzyme binding	identical protein binding
nitric-oxide synthase regulator activity	protein tyrosine kinase activity
ubiquitin protein ligase binding	protein kinase activity
estrogen receptor binding	protein kinase binding
transcription coactivator binding	kinase activity

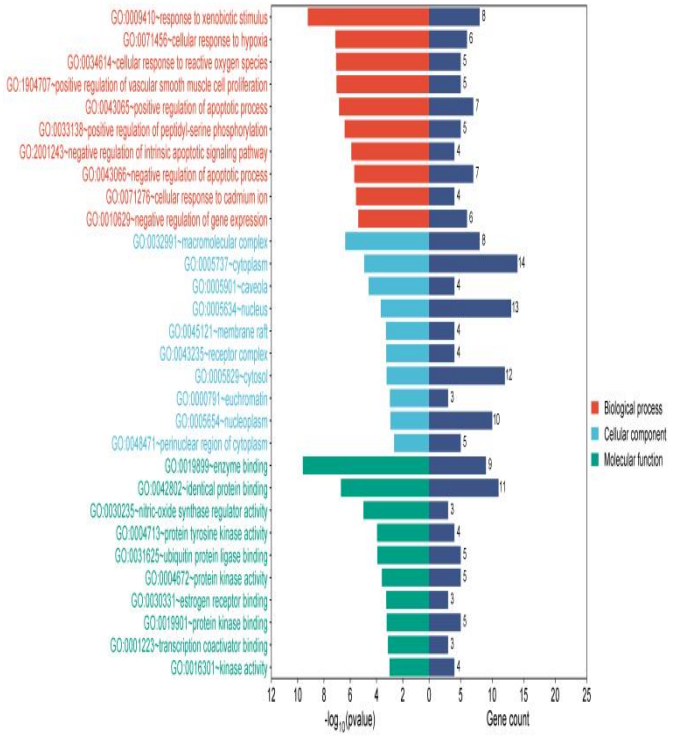


Fig 5. Go enrichment for the cluster analysis of the pathways

KEGG pathway enrichment was based on the criterion of

P<0.01. A total of 71 signaling pathways were screened, and the top 20 pathways with smaller P values and related to acute respiratory distress syndrome were selected for visual analysis. The P value represents the significance of the enriched target. The smaller the P value, the redder the color. The larger the P value, the greener the color. The size of the circular area indicates the amount of core protein involved; the larger the area, the more protein is involved. Among the top 20 pathways that conform to statistics are: HIF-1 signaling pathway, MicroRNAs and cancer, lipid and atherosclerosis, endocrine resistance and other signaling pathways, as shown in Figure 6.

A-Endocrine resistance	**B**-Pathways in cancer
C-Proteoglycans in cancer	**D**-Bladder cancer
E-Estrogen signaling pathway	**F**-EGFR tyrosine kinase inhibitor resistance
G-Prostate cancer	**H**-MicroRNAs in cancer
I-Lipid and atherosclerosis	**J**-Platinum drug resistance
k-Hepatitis B	**L**-AGE-RAGE signaling pathway in diabetic complications
M-Kaposi sarcoma-associated herpesvirus infection	**N**-HIF-1 signaling pathway
O-Chemical carcinogenesis - receptor activation	**P**-Human cytomegalovirus infection
Q-Thyroid hormone signaling pathway	**R**-Relaxin signaling pathway
S-Gastric cancer	**T**-Efferocytosis

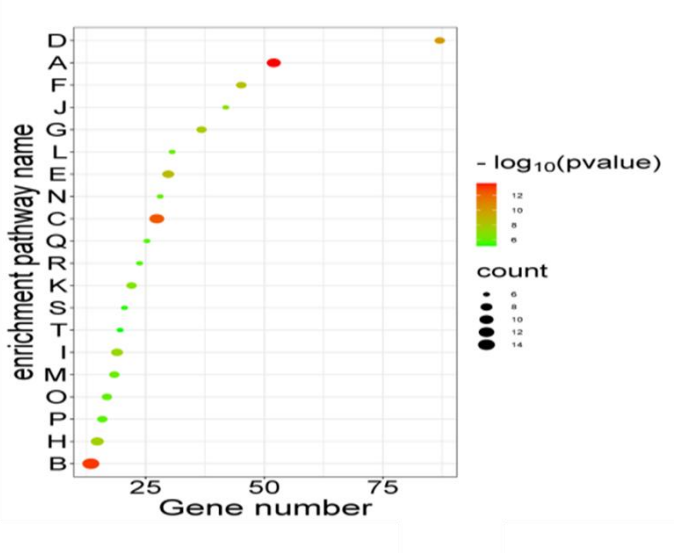


Fig. 6 Bubble dot diagram Of enriched KEGG pathways statistics

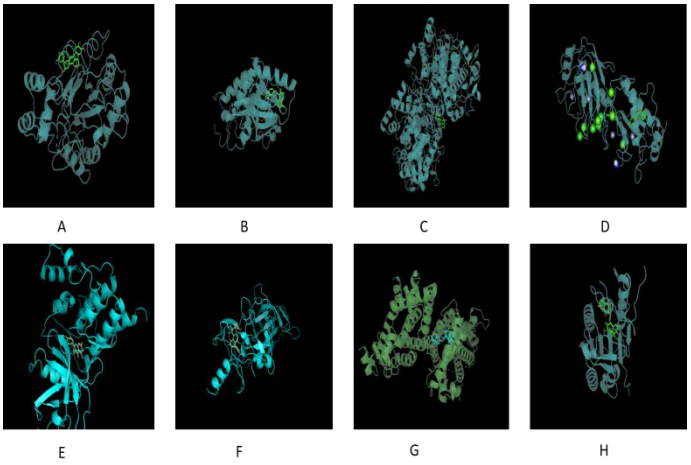
2.8 Molecular Docking

The core target proteins were subjected to molecular docking with the top 5 active components corresponding to loquat leaves with the highest degree values. The core protein ids were downloaded. Since ABCB1 had no core proteins that conformed to Homo sapiens and X-RAY, it was excluded. The docking results are shown in Table 3 and Figure 7. The docking score can evaluate the binding strength between the target and the active ingredient. The higher the absolute value of the score, the stronger the binding ability of the target to the active ingredient of the drug.

Table 3 .Molecular docking scores of active ingredients and target proteins

Active Ingredient	Target Protein	PDB ID	Docking Score
Isorhamnetin	ERBB2	8a27	-8.5
Isorhamnetin	JAK2	8bxh	-8.9
Kaempferol	MMP2	7xjo	-9.4
Kaempferol	MDM2	3iux	-8.2
Kaempferol	MMP9	8k5y	-8.6
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyra	PTGS2 (COX-2)	5f19	-9.6

no[6,5-h]chromen-2-one (Cinchonain 1c)			
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	SRC	2i16	-12.1
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	BCL2	8hts	-8.4
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	ESR1 (ER-α)	3cbp	-8.8
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	HIF1A	3hqu	-6.8
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	AKT1	8r5k	-7.4
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	APP (Amyloid precursor protein)	7zqx	-8.5
(4R,8R,9R)-4,8-bis(3,4-dihydroxyphenyl)-5,9-dihydroxy-4,8,9,10-tetrahydro-3H-pyrano[6,5-h]chromen-2-one	MAPK3 (ERK1)	7nrb	-7.7
7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin (Farnesiferol A)	EGFR	8a2d	-9.9
7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin	CASP3	4jje	-8.6
7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin	PPARG	8b94	-8.7
7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin	MAPK3 (ERK1)	7nrb	-7.7
7-[[(1S,4aS,6R,8aR)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decalin-1-yl]methoxy]coumarin	AKT1	8r5k	-7.4



A-cinchonatin 1c and non-receptor tyrosine kinase (SRC); B-farnesiverol and epidermal growth factor receptor (EGFR); C-cinchomycin 1c and prostaglandin peroxide synthase (PTGS2) 2; D-kaempferol and matrix metalloproteinase II(MMP2); E-isorhamnosin and non-receptor tyrosine protein kinase gene (JAK2) F-cinchonatin 1c and estrogen receptor 1 (ESR1) G-fannisivorol A and peroxisome proliferator-activated receptor -γ (PPARG); H-farnesiflor A and cysteine protease 3 gene (CASP3)

Fig. 5 Active components are docked with core targets

3. Discussion

Acute lung injury is the damage of pulmonary inflammatory response and alveolar-capillary barrier, resulting in pulmonary edema and hypoxemia. Its pathogenesis is complex and the treatment is difficult. Studies suggest that the extract of loquat leaves can counteract phenomena such as apoptosis during acute lung injury. This extract can stimulate the activity of the PI3K/Akt signaling pathway, thereby reversing the cell proliferation obstruction caused by LPS and the cell death resulting from oxidative stress, and alleviating the cell

damage caused by LPS [14]. The pentacyclic triterpenoids rich in loquat leaf extract have a significant inhibitory effect on neutrophil elastase in vitro and have shown therapeutic effects on acute lung injury in LPS-induced mouse models in vivo. This may be one of the potential mechanisms of loquat leaf in treating lung inflammation. [15-17]

This study utilized the network pharmacology method to explore the key active components, main action targets and core action network of loquat leaves in the treatment of acute respiratory distress syndrome. Through GO and KEGG enrichment analyses, the biological functions of these targets and their potential roles in disease treatment were further clarified, as well as the pharmacological mechanism of the active components in loquat leaves on acute lung injury. These findings provide research directions and theoretical support for future pharmacological experiments. Among the active components of loquat leaves, quercetin, kawhol, cinchonatin 1c, isorhamnetin and fanisifolol A have numerous connection nodes in the PPI network and score relatively high in molecular docking, which can improve acute lung injury. Isorhamnetin has the effects of anti-inflammation [18], antioxidation, anti-tumor [19], protection of neurons [20], and improvement of liver function [21]; Quercetin has a wide range of pharmacological activities, including anti-inflammatory, anti-tumor, antioxidant, anti-fibrotic, anti-allergic, anti-apoptotic and antiviral effects [22]; Kaempferol can exert anti-inflammatory, antioxidant and atherosclerotic functional effects, all of which belong to the core active ingredients for the treatment of ARDS. Cinchonasin 1c and fanicifol A have many linked nodes in the PPI network. However, no related studies have been found so far, and their pharmacological effects against ARDS need to be further explored and studied in depth.

3.1 Analysis of Protein Interaction Networks

Through the screening process of network research, we identified 17 core targets, including EGFR, Hypoxia-inducible factor 1-α (HIF1A), MMP9, MAPK3, PTGS2, etc., to evaluate the joint participation of the above proteins in regulating the treatment of ARDS. ARDS is a rapidly occurring alveolar injury and inflammatory response. EGFR is a transmembrane receptor that may be related to the injury and repair of alveolar epithelial cells. The activation of EGFR can stimulate the generation of inflammatory cytokines, leading to inflammatory responses. Studies by Shan, Xiaoou, etc. have shown that in vivo, in rat models treated with oral AG1478 and 451 for intrattracheal infusion of LPS, these inhibitors were observed to be able to block the activation of EGFR signaling in lung tissue. And it is helpful to alleviate inflammatory cell infiltration, inflammatory gene expression and lung injury caused by lipopolysaccharide [23]. HIF-1α is an important transcription factor and plays a crucial role in the survival and adaptation of cells under hypoxic conditions. In ARDS, severe lung injury leads to impaired oxygen exchange, causing hypoxemia. This hypoxic state activates HIF-1α. HIF-1α can activate NF-κB. The activation of NF-κB can promote the expression of various pro-inflammatory cytokines, including tumor necrosis factor -α (TNF-α), IL-1β, and IL-6. When they are overexpressed and released, it may trigger a cytokine storm, which is an abnormal and excessive immune response. It can aggravate the pathological process of ARDS and lead to further lung injury [24]. HIF1A may also affect the development of ARDS by regulating autophagy [25]. By regulating the activity of HIF-1α, it may have potential

therapeutic effects on controlling inflammation and improving hypoxic conditions. MMPs can induce pulmonary inflammation and increased endothelial barrier permeability in ALI/ARDS. During acute lung injury, the IL-33 / STAT3 / MMP2/9 signaling pathway in alveolar macrophages is directly reactivated, which may further intensify the inflammatory response in the lungs [26]. Another important core target protein in the PPI network is MAPK3. Studies have shown that abnormal DNA methylation in lung tissue may be related to the pathophysiology of LPS-induced ALI/ARDS. Three genes (Mapk3, Pak1 and Rac2) were verified in the control group of ALI and lung tissue by RT-PCR. It indicates that MAPK3 may be involved in the inflammatory response in ARDS [27]. In this study, PTGS2 is also one of the core proteins in the PPI network and is also known as Expression of cyclooxygenase-2 (COX-2). Its expression increases in ARDS and participates in regulating the inflammatory response. It is a metabolproduct of COX-2. In particular, Prostaglandin D2 (PGD2) synthesized by Lipoxygenase (L-PGDS) can promote the regression of inflammation. PGD2 exerts its effect by binding to its receptor DP1, which helps to alleviate inflammation and promote tissue repair [28]. Early studies by Mittal, Neha, et al. found that the increase in PTGS2 activity was associated with inflammatory responses and lung dysfunction, and inhibiting its activity might help improve the clinical symptoms of ARDS induced by LPS [29]. Analysis of the protein-protein interaction network indicates that loquat leaves achieve this in the treatment of acute respiratory distress syndrome by regulating inflammation, autophagy, and regulating signaling pathways.

3.2 Enrichment Analysis

Studies have found that MicroRNAs and pathways such as cancer, estrogen signaling pathway, HIF-1 signaling pathway, endocrine resistance, lipids and atherosclerosis are also involved in the mechanism of action of active components from loquat leaves in the treatment of ARDS. It involves endocrine metabolism, cancer, inflammatory response, lipid metabolism, etc. From this, it is speculated that the active components in loquat leaves may inhibit the pathological process of ARDS through the above pathways. The KEGG enrichment analysis in this study showed that MicroRNAs were significantly different from cancer and estrogen signaling pathways. Studies have shown that specific mirnas play an important role in the pathogenesis of ARDS. The research results of Xu et al. indicated that circulating serum miR-92a might be a risk factor for sepsis-induced ARDS. Inhibiting miR-92a could weaken the adverse effect of LPS on ARDS through the Akt/mTOR signaling pathway [30]. Furthermore, the down-regulation of miR-181a protects mice from damage induced by LPS by targeting Bcl-2 [31]. Studies have found that estradiol (E2) can effectively alleviate acute lung injury caused by seawater inhalation in rats by down-regulating AQP1 and AQP5 [32]. Doucet et al. 's research also indicates that estrogen can alleviate intestinal and lung injuries caused by early traumatic hemorrhagic shock, and its protective effect is mediated by the activation of ERα (estrogen receptor α), ERβ (estrogen receptor β), or both receptors [33]. The HIF-1 signaling pathway is an important way involved in immune responses and inflammatory reactions. In this study, KEGG analysis revealed significant differences in the HIF-1 signal transduction pathway, indicating that hypoxia-inducible factors play an important role in the treatment of ARDS with loquat leaves. Its mechanism of action may involve the aggregation of immune cells at the

inflammatory site. These areas often lead to a hypoxic environment due to insufficient oxygen supply. Under hypoxic conditions, PI-3K and MAPK/ERK are jointly activated, thereby stimulating the expression of HIF-1α in immune cells. NF-κB, as a key regulatory factor in the immune response, enhances the function of HIF-1α during the inflammatory process by positively regulating the expression of HIF-1α. This regulation may enhance the anti-inflammatory effect of loquat leaves in the treatment of ARDS [24, 34, 35]. Nf-kb regulates the expression of MMPs genes related to inflammation, immune response and tissue remodeling. Therefore, there should be a cross-talk among these pathways to play a role in improving ARDS. Combining the results of pathway enrichment analysis with the results of protein interaction suggests that loquat leaves can play an effective role in the process of acute lung injury through multiple signaling pathways.

3.3 Molecular Docking

The results of this study show that the binding abilities of the main active components of loquat leaves, such as isorhamnetin, kavelol, cinchonatin 1c, and Farnesifol A, with 16 core target proteins are all ≤-6.8, indicating strong binding activity. Among them, the docking score of cinchonasin 1c with 8 core proteins was relatively high, and its binding to SRC was as low as -12.1, with a very strong affinity. Isorhamnetin has a wide range of pharmacological activities, including effective anti-inflammatory effects. Its mechanism may be related to signaling pathways such as PI3K/AKT/PKB, NF-κB, MAPK, as well as the expression regulation of cytokines and kinases [36]. In the treatment of acute lung injury, studies have shown that isorhamnetin has a alleviating effect on acute lung injury in rats caused by hyperoxic conditions, and the mechanism may be related to the inhibition of the TLR4/NF-κB pathway [37]. Based on the enriched main signaling pathways, it is speculated that isorhamhamine in loquat leaves can be the main active substance for alleviating acute lung injury. Moreover, isorhamhamine down-regulates or inhibits inflammatory pathways and functions through signaling pathways such as lipid metabolism and inflammatory pathways, thereby exerting anti-inflammatory, antioxidant, and anti-apoptotic effects. Kaempferol has received considerable attention at present because it maintains extremely strong biological activities in terms of anti-inflammation, antioxidation and cancer prevention. Combined with the research results, kathol, by inhibiting the mRNA expression of pro-inflammatory factors NF-κB, IL-1β, HO-1, PTGES, iNOS, TNF-α and COX-2 in lung tissue, is conducive to improving the mRNA expression efficiency of anti-inflammatory factor HO-1. And it maintained a very strong protective effect in LPS-induced acute lung injury in mice [38]. This is consistent with the results of this study. In this study, the molecular docking scores of cinchonasin 1c and Farnesiflor A were relatively high. Among them, cinchonasin 1c had the highest docking score with 8 core targets, and the score with SRC was as low as -12.1. The affinity was very strong, and it was predicted to have a strong anti-ARDS effect. Since no two related disease experimental studies were found, its pharmacological effect and whether it has the therapeutic effect of improving ARDS need to be verified by further experiments. In terms of safety assessment, the research by Yi et al. showed that the maximum tolerated dose of loquat leaf extract for mice exceeded 10.0 (g/kg·bw). Moreover, no positive results were found in the three genotoxicity tests (Ames test, bone marrow cell micronucleus test and sperm abnormality test),

and there were no abnormalities in the physiological indicators of the test animals in the 90-day feeding test [39]. It can be confirmed that the intake of loquat leaves at the usual dose is safe.

In conclusion, natural products are increasingly favored due to their natural components and biological activities, as well as relatively few side effects, and are widely studied for the treatment of various diseases. The current research covers animal experiments, in vitro experiments and human clinical trials. These studies are constantly accumulating evidence to support the diverse health benefits of loquat leaves and their extracts. This study predicted that the active components of loquat leaves could regulate the inflammatory response through KEGG pathway enrichment and molecular docking methods. By regulating the activation of the HIF-1 signaling pathway and further regulating signal transduction pathways such as NF-κB and MAPK, it controlled the inflammatory response and improved the hypoxic state to exert an anti-ARDS effect. It reflects the characteristics of multiple components, multiple targets and multiple pathways of loquat leaves, providing a new perspective for in-depth research on its potential in the treatment of ARDS. These characteristics not only indicate the application prospects of loquat leaves in therapeutic strategies, but also open up new paths for the development of characteristic agricultural resources and the creation of new drugs in the southwestern region of our country. Although network pharmacology and molecular docking techniques provide a theoretical basis for exploring the mechanism of drug action, the inherent limitations of these methods and the limitations of existing databases may lead to uncertainties in the prediction results. Therefore, the relevant mechanisms for analysis and prediction need to be verified and supplemented through experimental research. Based on the prediction that the active components of loquat leaves have anti-ARDS effects, this study intends to conduct relevant verification experiments on cells and animals, expecting to make breakthroughs in exploring the molecular mechanism of natural drugs in anti-acute respiratory distress syndrome.

Conflict of Interests statement

None.

Conflict of funding statement

None.

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