



Network pharmacology and phytochemical composition combined with validation *in vivo* and *in vitro* reveal the mechanism of platycodonis radix ameliorating PM2.5-induced acute lung injury

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ABSTRACT

Ethnopharmacological relevance: Platycodonis radix (PR), the root of *Platycodon grandiflorus* (Jacq.) A. DC., is a traditional Chinese medicine recognized for its dual role as both a medicinal and dietary substance, exhibiting significant anti-inflammatory properties. It is frequently utilized in the treatment of lung diseases. However, the molecular mechanisms by which PR exerts its effects in the treatment of acute lung injury (ALI) remain unclear.

Aim of the study: This study presents a novel strategy that integrates network pharmacology, molecular docking, untargeted metabolomics analysis and experimental validation to investigate the molecular mechanisms through which PR treats ALI.

Materials and method: Initially, the bioactive components of PR, along with its targets and pathways in the treatment of ALI, were identified using network pharmacology. Following this, preliminary validation was conducted through molecular docking. The active ingredients in the aqueous extract of PR were characterized using HPLC-MS. Finally, *in vivo* and *in vitro* experiments were performed to further validate the findings from the network pharmacology.

Results: A total of 14 bioactive components and 156 effective targets were identified using the TCMSP, DisGeNET, Genecard, OMIM databases and Venny 2.1.0. Protein-protein interaction (PPI) analysis revealed 22 core targets including TP53, AKT1, STAT3 and JUN. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses indicated that these targets primarily participate in the regulation of cellular apoptosis, lung cancer and inflammatory pathways. Molecular docking demonstrated that four bioactive components exhibited strong affinities with their respective docking targets. LC-MS analysis confirmed that the aqueous extract of PR contained 87 components, including two active ingredients identified through network pharmacology and molecular docking. Preliminary validation was conducted in mice with ALI induced by acute PM2.5 exposure, revealing that the aqueous extract of PR reduced inflammatory factor levels in bronchoalveolar lavage fluid, enhanced antioxidant capacity in lung tissue, and decreased lung cell apoptosis in PM2.5-exposed mice. Notably, PR alleviated PM2.5-induced ALI through the STAT3, JUN, and AKT1 signaling pathways.

Abbreviations: ALI, acute lung injury; TCM, Traditional Chinese Medicine; PR, platycodonis radix; PMs, particulate matter; PM2.5, particulate matter with a diameter smaller than 2.5 μm ; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; PG, *Platycodon grandiflorus* (Jacq.) A. DC.; ELISA, Enzyme-linked immunosorbent assay; MDA, malondialdehyde; CAT, catalase; MPO, myeloperoxidase; SOD, superoxide dismutase; TUNEL, Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling; OB, oral bioavailability; DL, drug-likeness; PPI, protein-protein interaction networks; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NCE, Normalized collision energy; PBS, phosphate balanced solution; BALF, bronchoalveolar lavage fluid; H&E, hematoxylin-eosin; RIPA, Radio-Immunoprecipitation Assay; SD, mean $\pm$ standard deviation; ANOVA, analysis of variance; BP, biological processes; CC, cellular components; MF, molecular functions; LPS, lipopolysaccharides; TP53, Tumor protein P53; AKT1, Protein kinase B α ; STAT3, Signal transducer and activator of transcription 3; JUN, Activator protein 1; MAPK1, Mitogen-activated protein kinase 1.

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Similarly, the results of *in vitro* intervention experiments further confirmed that the aqueous extract of PR protected pulmonary epithelial cells against PM2.5 exposure through activating AKT1 signalling pathway, and inhibiting STAT3 and JUN signalling pathways.

Conclusion: This study identifies the active components of PR and elucidates the molecular mechanisms by which PR alleviates ALI, specifically by inhibiting the phosphorylation levels of STAT3 and c-JUN, or by activating the phosphorylation level of AKT1. These results provide a foundational basis for the application of PR in the treatment or prevention of lung injuries induced by particulate matter.

1. Introduction

Air pollution is one of the most critical threats to human well-being in the context of accelerated societal modernization. Statistics indicate that air pollution is responsible for up to 7 million deaths worldwide each year (Thangavel et al., 2022). Air pollutants can be classified into particulate matter (PM) and gaseous pollutants (Shahrbaef et al., 2021). The pathogenicity of PMs is influenced by their composition, source, size, and solubility. Particles with a diameter smaller than 2.5 μm , referred to as PM2.5, can penetrate the respiratory tract and enter the lungs, leading to a series of inflammatory reactions that impair lung function. Exposure to PM2.5 has detrimental effects on respiratory health, including acute lung injury (ALI), lung cancer and other lung diseases. ALI is a clinical syndrome associated with high morbidity and mortality rates and serves as an early manifestation of acute respiratory distress syndrome (ARDS) (He et al., 2021). The pathogenesis of ALI involves a cascade of inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), released by neutrophils, macrophages, endothelial cells and mast cells (Mokra, 2020). This cascade results in increased pulmonary microvascular permeability and diffuse damage to the alveolar capillary membrane. These pathological changes can lead to pulmonary edema and atelectasis, ultimately resulting in hypoxic respiratory insufficiency or respiratory failure (Mowery et al., 2020). Therefore, effective control of the inflammatory response may mitigate the detrimental effects of ALI. In summary, it is crucial to identify effective drugs or functional foods with high efficacy and minimal side effects to prevent or alleviate ALI, particularly that which is induced by PM2.5.

Currently, there is no specific treatment for ALI. The clinical therapies applied in ALI is mainly symptomatic therapy such as mechanical ventilation and fluid management (Liu et al., 2022). It is particularly noteworthy that, to date, scientists worldwide have not identified a specific drug for the treatment of ALI. However, as research advances, an increasing number of researchers are discovering that certain functional foods and herbal remedies may hold promise in the prevention and treatment of ALI. *Scutellaria baicalensis*, a traditional Chinese medicine, possesses significant medicinal value attributed to its root. Recent studies have demonstrated that baicalin, a compound found in the root, can inhibit the HMGB1/NLRP3/Caspase1 pathway. This inhibition leads to a reduction in the release of inflammatory cytokines, ameliorates pathological damage to lung tissue, and enhances respiratory function in mice exposed to PM2.5 (Deng et al., 2022). Similarly, extract from the root of *Adenophora stricta* Miq. was highly protective against PM2.5-induced ALI in mice (Park et al., 2022). With the introduction of the “lung-gut axis” theory, researchers have also tried to improve PM2.5-induced ALI through changes in gut flora. Wu found that probiotics may ameliorate PM2.5-induced lung inflammation by modulating gut microbiota and improving Th17/Treg imbalance (Wu et al., 2022). In addition, Modified Guo-Min decoction and Ma xing shi gan decoction could regulate the phosphorylation level of AKT to improve PM2.5-induced ALI, respectively (Wang et al., 2020, 2024). It is seen that traditional Chinese medicine has a promising future in the treatment of PM2.5-induced ALI.

Platycodon grandiflorus (Jacq.) A. DC. (PG) belongs to the family Campanulaceae (Zhang et al., 2020) and is widely cultivated in north-eastern Asia. *Platycodonis radix* (PR), the root of PG, is recognized as a

medicinal and food homology herb. It is rich in vitamin B1, vitamin C and saponin compounds, and has the potential to be developed into a beverage and food additive (Zhang et al., 2022). Importantly, PR is known for its ability to relieve lung congestion and alleviate sore throat, and it has been effectively used as a treatment for bronchitis, laryngitis, and tonsillitis in Northeast Asia, including China, Korea, and Japan, according to the Committee for the Pharmacopoeia of PR China (2020). Previous studies have demonstrated that PR exhibits anti-inflammatory, hepatoprotective, anti-tumor, renal protective, and immune function activation properties (Zhang et al., 2015). However, the potential molecular mechanisms underlying the protective effects of PR against PM2.5-induced ALI remain poorly understood.

Network pharmacology, an innovative approach that integrates pharmacology, systems biology, and computational analytical techniques, is employed to elucidate the complex relationships among the components of traditional Chinese medicine (TCM) components, diseases and targets (Jiashuo et al., 2022). This field facilitates the understanding of the biological processes and pathways through which TCM addresses diseases by analyzing the interplay between the bioactive components of TCM and their associated targets. Consequently, it aids in exploring the mechanisms underlying TCM's therapeutic effects and offers valuable directions for practical research.

In this study, we constructed a network diagram illustrating the relationships between the bioactive ingredients of PR, their targets, and ALI. We identified the potential bioactive ingredients and their corresponding core targets of PR against ALI through network pharmacology and molecular docking. Additionally, we characterized the chemical profile of PR extracts using LC-MS, and the molecular mechanism by which PR extracts alleviate PM2.5-induced ALI was validated through *in vivo* and *in vitro* experiments. This work provides new insights into the potential mechanisms by which PR may prevent or mitigate ALI induced by particulate matter.

2. Materials and methods

2.1. Materials and reagents

PM2.5 was purchased from NIST (MD, USA) and *platycodonis radix* was obtained from Anguo Herbal Base (Hebei, China). MK2206, Anisomycin and Colivelin were purchased from MCE (New Jersey, USA). L-1 β , IL-6 and TNF- α enzyme-linked immunosorbent assay (ELISA) kits were purchased from ZCI BIO (Shanghai, China). Malondialdehyde (MDA) and catalase (CAT) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Myeloperoxidase (MPO) and superoxide dismutase (SOD) activity assay kits were achieved from Solarbio (Beijing, China). TUNEL apoptosis assay kit was purchased from Beyotime (Shanghai, China). Antibodies against the following targets were purchased from HUABIO (Hangzhou, China) and Santa Cruz Biotechnology (SantaCruz, CA, USA): STAT3 (ET1607-38), p-STAT3 (ET1603-40), AKT1 (ET1609-47), p-AKT1 (ET1607-73), c-JUN (sc-74543), p-c-JUN (sc-822) and β -actin (sc-47778). The secondary antibodies including IRDye-680LT goat anti-rabbit IgG and IRDye-800CW goat anti-mouse IgG were provided by LI-COR Inc. (Lincoln, NE, USA).

2.2. Screening the bioactive ingredients in PR by network pharmacology

The primary bioactive components of PR were screened and obtained by the TCMSP database, based on two key criteria: drug-likeness (DL) ≥ 0.18 and oral bioavailability (OB) $\geq 30\%$ (Zhu et al., 2021). Additionally, the constituents of PR that have undergone pharmacological activity studies were sourced from the PubMed database. The bioactive components of PR screened from different databases were de-duplicated to obtain the final bioactive components.

2.3. Screening PR's targets and constructing PR-components-targets network

The relevant targets of the bioactive components of PR were identified by searching for *Platycodon grandiflorus* in the TCMSP database. Additionally, target predictions for active ingredients whose targets were not found in the TCMSP database, were performed using the Swiss TargetPrediction database (Tan et al., 2022). The canonical SMILES of the bioactive ingredients obtained from the PubChem database were then entered into the Swiss TargetPrediction database to visualize their targets. Targets of the retrieved bioactive ingredients were de-duplicated and then standardized in the Uniprot database, limited to the species "Homo sapiens (Human)". Eventually, the bioactive components of PR and their corresponding targets were imported into Cytoscape 3.8.0 software to establish the PR-components-targets network.

2.4. Collecting targets for ALI

The targets of ALI were collected by searching the keywords "Acute lung injury" or "ALI" in the DisGeNET, GeneCards and OMIM databases (Zu et al., 2021). Then, the targets of ALI were obtained by standardizing in Uniprot database with the limited species as "Homo sapiens (Human)" and de-duplicating.

2.5. Venn analysis and PR-components-targets-ALI network construction

The targets of bioactive compounds in PR and the targets of ALI were analyzed with Venny 2.1.0 to screen the shared targets as effective targets of PR in treating ALI. Then, the bioactive components of PR and shared targets were input into Cytoscape 3.8.0 software to construct the PR-bioactive components-shared targets-ALI network.

2.6. PPI network construction and analysis

The shared targets were inputted into the String database with the quantification of organism as "Homo sapiens", leading to the extraction of protein-protein interaction networks (PPI) through the search and analysis of effective targets for PR in the treatment of ALI (Szkłarczyk et al., 2023). Degree, Betweenness, Closeness and Eigenvector were obtained by plotting and analyzing the PPI in Cytoscape 3.8.0 software, and then the core targets were screened by making them greater than their respective averages.

2.7. GO and KEGG pathway enrichment analysis and constructing PR-components-core targets-pathways-ALI network

The core targets were inputted into the DAVID database to conduct enrichment analyses for GO and KEGG pathways. The results of the GO and KEGG pathway enrichment analyses were visualized in Bioinformatics software. Additionally, the interrelationship data among PR and its components, components and targets, pathways and targets, as well as ALI and pathways, were entered into Cytoscape 3.8.0 to construct the PR-compounds-core targets-pathways-ALI network.

2.8. Molecular docking

Based on the results of the PPI analysis, core targets with the top five of the Degree values were selected for molecular docking with the corresponding bioactive ingredients. Files in mol2 format for the energy minimization of the bioactive components were obtained from the TCMSP database. The three-dimensional chemical structures of the core targets, serving as protein receptors, were downloaded from the PDB database and imported into PyMOL 2.3.0 software for the removal of water and ligand molecules. The core target and its corresponding bioactive ingredients were then imported into AutoDock Tools software for pre-processing, converted into PDBQT format, and saved. The molecular docking process was conducted using AutoDock Vina software to identify the optimal binding site, which was subsequently visualized in PyMOL 2.3.0 software.

2.9. LC-MS analysis

2.9.1. Preparing the lyophilized powder of PR

A 25 g sample of PR powder was passed through a screen mesh with a pore diameter of 380 μm , then dissolved in 50 mL of ultrapure water and subjected to ultrasonic extraction for 60 min at 100 W and 80 Hz. The supernatant was collected by centrifugation at 8000 rpm and 4 °C for 10 min, and this procedure was repeated three times. All supernatants were concentrated in a rotary evaporator and then freeze-dried in a lyophilizer to obtain lyophilized powder of PR.

2.9.2. LC-MS analysis

The Ultimate 3000 UPLC and Q-Orbitrap MS were employed to analyze the composition of aqueous extracts of PR. The selected column model was HSS T3 (1.8 μm , 2.1 $\times$ 100 mm). The mobile phase consisted of water (containing 0.1% formic acid, A) and methanol (D). The optimized elution conditions were detailed as follows: 0–4 min, 0–3% D; 4–8 min, 3–23% D; 8–13 min, 23–23.3% D; 13–14 min, 23.3–34% D; 14–17 min, 34–34% D; 17–19 min, 34–36% D; 19–21 min, 36–55% D; 21–23 min, 55–70% D; 23–24 min, 70–80% D; 24–28 min, 80–100% D; 28–30 min, 100–100% D. The column temperature was maintained at 30 °C, with a flow rate of 0.3 mL/min and a sample injection volume of 10 μL . The primary ion source parameters were set as follows: electron spray voltage of 3.5 kV (+) and 3.0 kV (–), capillary temperature of 320 °C, auxiliary gas temperature of 350 °C. Normalized collision energy (NCE) was set to 30 V, and the acquisition mode of Full MS ddMS² was utilized.

2.10. Experiments in vivo

2.10.1. Animals

Male C57BL/6 mice (7–8 weeks old, weighing 18–20 g) were sourced from the National Institutes for Food and Drug Control in Beijing, China. Following a 7-day acclimatization period, the formal experiment commenced. The mice were randomly assigned to four groups (n = 8): Control, PM2.5, PM2.5 + PR-L (100 mg/kg), and PM2.5 + PR-H (200 mg/kg). Mice were treated with 100 μL PBS or PR by daily oral gavage. At the beginning of day 13, the control group mice received a 50 μL saline solution via the tracheal drip method, while the mice in the model and experimental groups were administered an equivalent volume of PM2.5 at a concentration of 5 mg/mL for three consecutive days.³ The mice were sacrificed on the 17th day, and then lung tissue and bronchoalveolar lavage fluid (BALF) were gathered for further experiments. Animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals and approved by the Animal Ethics Committee of Tianjin University of Science and Technology (No: 20221020CB).

2.10.2. Lung coefficient

Daily recordings of the body weight of mice were conducted, and the

weight of lung tissue was recorded at the conclusion of the experiment. The specific calculation method for the lung coefficient is defined as follows: Lung coefficient = (lung tissue weight (g)/body weight (kg)) × 100% (Cao et al., 2022).

2.10.3. ELISA analysis

The concentrations of inflammatory factors IL-1 β , IL-6, and TNF- α in BALF were assessed using ELISA kits specific for IL-1 β , IL-6, and TNF- α .

2.10.4. Histological analysis

A sample of lung tissue was extracted and immersed in 4% paraformaldehyde overnight. Following dehydration and transparency treatment, the lung tissue samples, enclosed in paraffin blocks, were sliced into 4 μ m sections utilizing a Leica RM216RT slicer. The lung slices were then stained with hematoxylin-eosin (H&E) to assess histopathological alterations. Eventually, the sections were observed with an Olympus BX53 orthogonal fluorescence microscope (Japan). The histopathological scoring of the lung sections was performed according to the following standards: a) degree of accumulation of neutrophils or vascular wall thickening, b) alveolar congestion, and c) hemorrhage or alveolar wall thickness. The scoring guidelines are as follows: 0 = absent, 1 = slight, 2 = moderate, 3 = intense, and 4 = extremely severe (Wang et al., 2022a).

2.10.5. Assay of oxidative stress indicators

Some lung tissue were taken and thoroughly ground on a grinder, followed by centrifugation. The supernatants were used for the analysis of MDA, SOD, CAT and MPO activities according to their instruction manuals.

2.10.6. TUNEL assay

Following the TUNEL operating instructions, lung tissue sections were cleaned, fixed, stained, and subsequently observed under an orthogonal fluorescence microscope (Olympus BX53).

2.10.7. Western blot

Lung tissues were lysed using RIPA lysis buffer, which included protease and phosphatase inhibitors at 4 °C for 30 min, and then lung tissue was thoroughly ground with a grinder. The protein samples obtained by the above operation were submitted to Western blot assay. Ultimately, protein bands were identified utilizing an Odyssey infrared laser imaging system (LI-COR, USA), and quantitative analysis was performed with ImageJ software.

2.11. Cell culture and stimulation

The human lung epithelial cell line BEAS-2B was purchased from FENGHUISHEGWU (Changsha, China) and cultured in DMEM medium (Thermo Fisher, Waltham, USA) with 10% fetal bovine serum, 5% CO₂ and 95% O₂ at 37 °C. To determine the impact of PM2.5 on cell survival, BEAS-2B cells were exposed to various concentrations of PM2.5, and cell counting was performed. To investigate the effect of PR extract on pulmonary epithelial cells exposed to PM2.5, cells were pre-incubated with different concentrations of PR extracts and then exposed to 200 μ g/mL of PM2.5 for another 24 h. Cell viability was assessed by MTT assay and the levels of inflammatory factors in the culture supernatant were detected by ELISA.

To further identify the key signaling pathways involved in the improvement of PM2.5-induced lung injury by PR extract, cells were pretreated with 200 μ g/mL of PR extracts for 24 h, and then incubated with 4 μ M of anisomycin (a JNK activator), 12.5 μ g/mL of colivelin (a STAT3 activator) or 50 μ M of MK2206 (a AKT inhibitor), followed by exposure to 200 μ g/mL of PM2.5 for another 1h. The protein samples were then collected for Western blot.

2.12. Statistical analysis

Graphpad Prism 8.0 software was used to perform statistical analysis on the experimental data. The data were presented as “mean $\pm$ standard deviation (SD)”. Comparisons of significance among multiple groups were conducted through one-way ANOVA. Significance levels were denoted as #P < 0.05, ##P < 0.01, ###P < 0.001, *P < 0.05, **P < 0.01 and ***P < 0.001 to demonstrate statistical significance of the results.

3. Results

3.1. Bioactive components of PR and their targets

A total of 102 ingredients of PR were found in the TCMSP database using the keyword “jiegeng”. Applying the criteria of “OB $\geq$ 30% and DL $\geq$ 0.18”, seven bioactive ingredients were selected: acacetin, spinasterol, 2-O-methyl-O- β -D-glucopyranosyl platycogenate A, cis-Dihydroquercetin, dimethyl 2-O-methyl-3-O- α -D-glucopyranosyl platycogenate A, luteolin and robinin. Based on the literature available in the PubMed database, 14 of these ingredients have been studied and are recognized for their pharmacological activity. Following the deduplication of ingredients from various databases, 16 distinct bioactive components of PR were obtained, as detailed in Table 1.

Utilizing the TCMSP database, a search was conducted to identify the targets of the bioactive components. In total, 112 targets associated with the bioactive constituents of PR were discovered within the database. For the bioactive ingredients whose targets were not available in the TCMSP database, the PubChem and Swiss TargetPrediction databases were employed to predict their potential targets, resulting in 228 predicted targets. However, the targets for 2-O-methyl-O- β -D-glucopyranosyl platycogenate A and dimethyl 2-O-methyl-3-O- α -D-glucopyranosyl platycogenate A were not found in these databases. After standardization and deduplication, a total of 167 targets for the bioactive ingredients of PR were obtained.

The bioactive ingredients of PR and their corresponding targets were analyzed using Cytoscape 3.8.0 software to construct the PR-ingredients-targets network. As shown in Fig. 1, the yellow triangles represent the drug, blue rhombuses indicate the bioactive ingredients, red circles indicate the targets, and black connecting lines indicate the relationships between the bioactive ingredients and their targets. The network contained 182 nodes and 377 edges. Notably, the degree values of platycodigenin, polygalacic acid, luteolin, acacetin and robinin were greater than 20, highlighting the significant role of these bioactive ingredients. This network diagram demonstrates that a single component can interact with multiple targets, while various components can target

Table 1
The bioactive components of PR.

Mol ID	Ingredients	OB/ (%)	DL
MOL001689	Acacetin	34.97	0.24
MOL004355	Spinasterol	42.98	0.76
MOL004580	Cis-dihydroquercetin	66.44	0.27
MOL005996	2-O-methyl-O- β -D-glucopyranosyl platycogenate A	45.15	0.25
MOL000006	Luteolin	36.16	0.25
MOL006026	dimethyl 2-O-methyl-3-O- α -D-glucopyranosyl platycogenate A	39.21	0.25
MOL006070	Robinin	39.84	0.71
MOL006024	Deapio-platycodin D	2.85	0.03
MOL006025	Deapio-platycodin D3	5.88	0.01
MOL006049	Platycodigenin	14.62	0.67
MOL006050	Platycodin D2	7.57	0.00
MOL006051	Platycodin D3	7.57	0.00
MOL006066	Polygalacic acid	20.07	0.70
MOL006072	Platycodin A	1.66	0.02
MOL006073	Platycodin C	1.61	0.02
MOL006074	Platycodin D	7.60	0.01

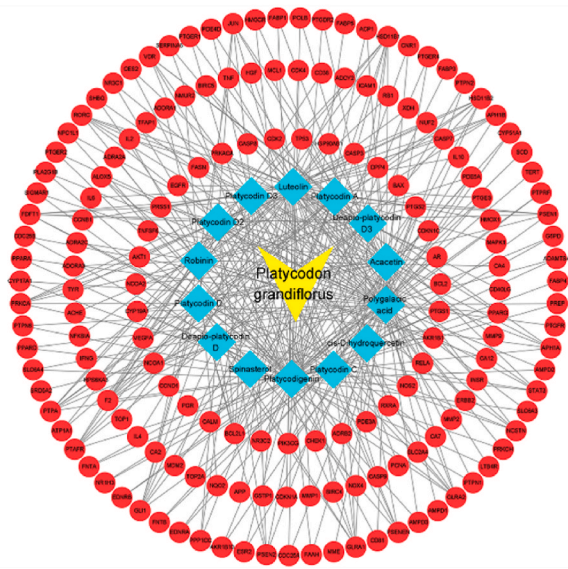


Fig. 1. The PR-compounds-targets network.

the same entity, indicating the potential of PR in treating diseases through multi-components and multi-targets.

3.2. Venn analysis and PPI network analysis

The targets of ALI were explored using the DisGeNET, GeneCards and OMIM databases. A total of 93 targets were received at the DisGeNET

database, 9512 targets were found in the GeneCards database, and 372 targets were taken from the OMIM database. After standardization and deduplication, a total of 9815 targets related to ALI were obtained.

The targets of the bioactive components of PR and the targets associated with ALI were analyzed using Venny 2.1.0 to screen shared targets that may serve as effective targets for PR in the treatment of ALI. As shown in Fig. 2A, 156 common targets were screened as effective targets for PR treatment of ALI, with their details provided in Table S1 (Supplementary Data). These shared targets were then input into Cytoscape 3.8.0 software to construct the PR-ingredients-shared targets-ALI network (Fig. 2B). In this network, red triangles represent the drug, yellow rhombuses indicate the bioactive ingredients, green squares indicate the targets, blue parallelogram means disease, and black connecting lines indicate the interrelationships among PR, bioactive components, targets and ALI. The network contained 165 nodes and 475 edges. The degree values of platycodigenin, polygalacic acid, and luteolin were 60, 60, and 53, suggesting that these bioactive ingredients may be key components in the treatment of ALI by PR.

The shared targets of PR in treating ALI were inputted into the STRING database to analyze interactions among proteins. The PPI network was obtained by screening the analysis results with a confidence level greater than 0.7 and removing unconnected targets, as shown in Fig. 2C. The PPI network diagram contained 134 nodes and 1392 edges with an average degree values of 20.77. The network nodes represent target proteins, and the lines represent the connections between them. The averages of degree value, betweenness value, closeness value, and eigenvector value of effective targets were 20.77, 214.38, 0.1745, and 0.05875, respectively. A total of 22 effective targets were screened as core targets based on these average values, and a histogram of the core targets was plotted (Fig. 2D). Among these, the top five core targets ranked by degree values were TP53, AKT1, STAT3, JUN, and

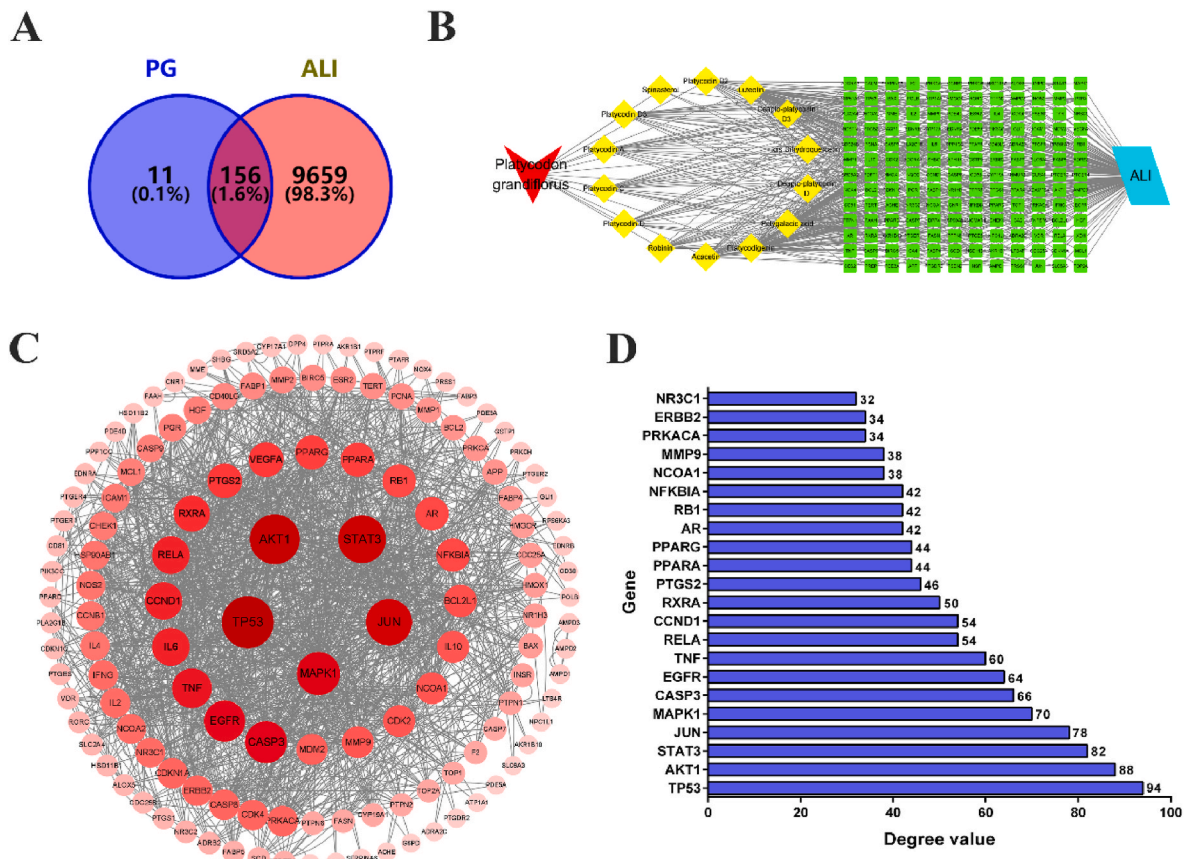


Fig. 2. Screening of core targets of PR in the treatment of ALI. (A) Venn Diagram of PR and ALI related targets. (B) The PR-components-common targets-ALI network. (C) PPI network diagram. (D) Histogram of the core targets.

MAPK1, with degree values of 94, 88, 82, 78, and 70, respectively.

3.3. GO and KEGG pathway enrichment analysis and network analysis

The core targets of PR and ALI were submitted to the DAVID database for relevant GO and KEGG pathway enrichment analyses. The results revealed a total of 303 GO functional entries, which included 225 biological processes (BP), 23 cellular components (CC) and 55 molecular functions (MF). From these, 187 BP, 17 CC, and 46 MF were selected based on a stringent criterion ($P < 0.05$). The top 10 enrichments with the smallest P-values in each category were chosen for visualization, and the results were represented using a bioinformatics online platform. The BP enrichment analysis highlighted processes such as response to drug, positive regulation of apoptotic process, negative regulation of apoptotic regulation and positive regulation of cell proliferation. The CC comprised membrane raft, macromolecular complex and cytosol, and the MF contained enzyme binding, protein binding and identical protein binding (Fig. 3A). The KEGG pathway enrichment analysis showed 133 signaling pathways, and 124 signaling pathways were obtained by screening the results with a limited condition ($P < 0.05$). KEGG pathways of enrichment analysis included pathways in cancer, non-small cell lung cancer, small cell lung cancer, TNF signaling pathway, Epatein-Bar

virus infection, among others (Fig. 3B). Most of these pathways contained several key targets including STAT3, AKT1, JUN, and TP53.

To delve deeper into the potential mechanisms of PR in treating ALI, the bioactive components of PR, core targets, and the 20 pathways with the smallest P-values were entered into Cytoscape 3.8.0 software to establish the PR-components-core targets-pathways-ALI network diagram (Fig. 3C). In this diagram, yellow similar-triangles represent the drug, blue diamonds indicate the bioactive ingredient, orange squares indicate the target, yellow triangles show the pathways, purple parallelograms mean disease and black connecting lines indicate the interrelationships among PR, bioactive components, targets, pathway and ALI. The network diagram included 57 nodes and 297 edges. These analyses indicated that PR treatment for ALI exhibits characteristics of multi-component, multi-target and multi-pathway.

3.4. Molecular docking

Based on the results of PPI, the top five targets and their corresponding bioactive components were selected for molecular docking. TP53, AKT1, MAPK1, STAT3 and JUN were docked 50 times with acetin, luteolin, deapio-platycodin D, and platycodin D, individually. The combination with the lowest binding energy was selected for the

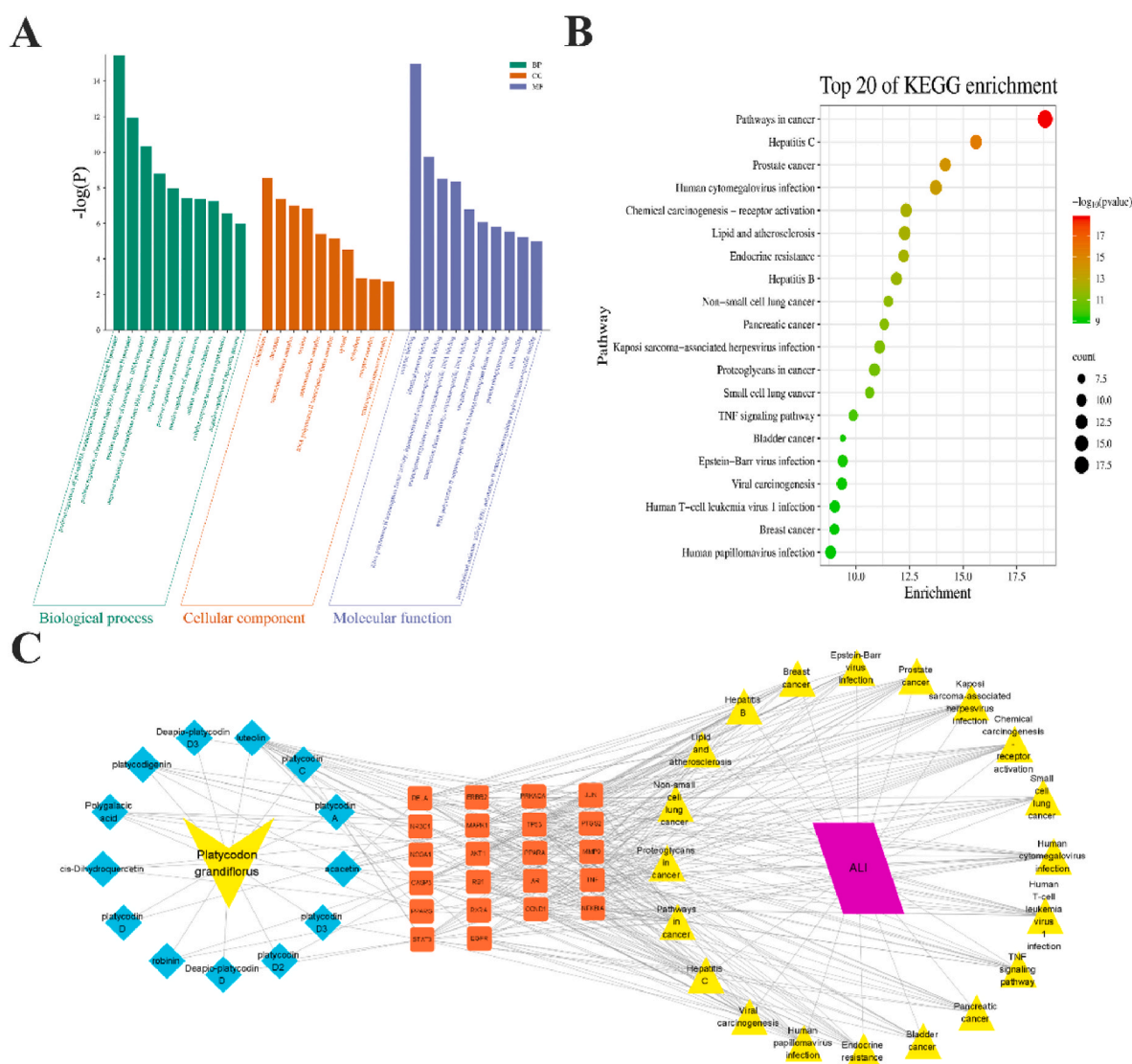


Fig. 3. Enrichment analysis of core targets. (A) Bar graph of top 10 of biological processes, cellular components or molecular functions. (B) The top 20 of KEGG pathway enrichment analysis of core targets. (C) The PR-components-core targets-pathways-ALI network.

analysis of molecular docking. The binding energies of the targets and their bioactive components are shown in Table 2. The minimum binding energies of all docking combinations were below -7 kcal/mol, suggesting that the intermolecular forces between the drug molecule and its target were strong (Xu et al., 2021). These results also revealed that platycodonis radix might exert its therapeutic effect on ALI mainly through these 4 pharmacodynamic components and the corresponding 5 targets.

To enhance comprehension of the binding sites between the bioactive components of PR and five corresponding targets, the results were analyzed by PyMol 2.3.0 software (Fig. 4). The results of the docking show that acacetin forms a total of 5 hydrogen bonds with ARG-19, ASN-182, ASN206 and CYS-181 on TP53, luteolin forms a total of 6 hydrogen bonded with the GLN-79, ASN-54, THR-211 and SER-205 of AKT1, deapio-platycodin D forms a total of 11 hydrogen bonded with the GLN-326, GLN-247, ALA-250, ASP-334, THR-515, LYS-573 and SER-514 on STAT3, and platycodin D forms a total of 5 hydrogen bonded the DA-214, DG-308, DA-309, DG-211 and DC-313 on JUN. Therefore, these several bioactive components of PR might be effective in the treatment of ALI by affecting the function of these five target proteins.

3.5. The main components of platycodonis radix aqueous extract by LC-MS analysis

The results showed that 87 ingredients of platycodonis radix were analyzed by the LC-MS (listed in Table S2 in Supplementary Data). As shown in Table S3 and Fig. 5, eight types of platycodon saponin components, including platycodin A, platycodin C, platycodin D, platycodin D2, platycodin D3, deapio-platycodin D, deapio-platycodin D2 and deapio-platycodin D3, have been detected in the water extract of platycodonis radix. These components have been reported in studies and can be considered as bioactive constituents of platycodonis radix. Acacetin and luteolin were not detected in the water extract of platycodonis radix, which could be due to their lipophilic characteristics.

3.6. The aqueous extract of PR alleviated PM2.5-induced ALI by decreasing the levels of the inflammatory factors

To further make sure the impact and molecular mechanism of PR in mitigating ALI induced by PM2.5, preliminary validation was carried out in ALI-afflicted mice following acute exposure to PM2.5 (Fig. 6A). The severity of pulmonary edema in PM2.5-treated mice was notably higher, resulting in a significant increase in lung coefficient compared to the control group. The PM2.5-induced lung coefficient elevation was suppressed by the PR treatment relative to the model group (Fig. 6B). Subsequently, the expression levels of the inflammatory factors IL-1 β , IL-6 and TNF- α in BALF were analyzed by ELISA, and these results were shown in Fig. 6C–E. The levels of IL-6, IL-1 β , and TNF- α were significantly raised in the model group compared to the control group, while both the low-dose and high-dose groups of PR inhibited the secretion of inflammatory factors relative to the model group. A portion of lung tissue was taken for histopathological analysis. As shown in Fig. 6F, in the control group, the alveolar size was not altered, the capillaries in the lung tissue were not enlarged, and the phenomenon of hemorrhage and the infiltration of inflammatory cells in the alveolar lumen were not

detected. Black particles could be observed on the bronchial wall of the model group, with alveolar hemorrhage and inflammatory cell infiltration, and thickening of the alveolar wall. The pathological impairments were attenuated in the low-dose and high-dose PR groups. Nevertheless, there are still some pathological phenomena, such as hemorrhage, inflammatory cell infiltration, and widened alveolar walls.

3.7. The aqueous extract of PR alleviated PM2.5-induced ALI by reducing oxidative stress and cell apoptosis

ALI is closely linked to oxidative stress, with PM2.5 capable of triggering oxidative stress and subsequently exacerbating lung disease progression (Mazzoli-Rocha et al., 2010). Here, the oxidative stress indicators MDA, MPO, SOD and CAT were assayed. As shown in Fig. 7A, the MDA levels were low in the control group, whereas PM2.5 treatment induced more MDA production. PM2.5 treatment also increased the levels of MPO and inhibited the levels of SOD and CAT (Fig. 7B–D). When mice were gavaged with different concentrations of PR, these tendencies caused by PM2.5 were reversed. Furthermore, TUNEL analysis confirmed that PM2.5 could induce abundant lung cell apoptosis compared to the control group, while the apoptotic cells were significantly reduced after PR treatment (Fig. 7E).

3.8. The aqueous extract of PR alleviated PM2.5-induced ALI via AKT, STAT3 and JUN signal pathways

Network pharmacology and molecular docking revealed that AKT, STAT3 and JUN could be key targets of PR in alleviating ALI induced by PM2.5. In order to verify the results, the expressions of p-STAT3 and p-JUN were detected in the lung tissue of PR-treated mice in response to PM2.5 exposure by Western blot. The results indicated that PR supplementation significantly inhibited the high level of p-STAT3/STAT3 induced by PM2.5 (Fig. 8A and B). PM2.5 exposure could down-regulate the level of p-AKT1/AKT1, which was reversed by high-dose PR. Furthermore, PR treatment could inhibit the PM2.5-induced increase in the protein expression of both p-JUN and JUN, as shown in Fig. 8C. These results indicated that PR could inhibit lung cell apoptosis and the increase of inflammatory factors by activating the AKT1 signaling pathway and suppressing the STAT3 and JUN signaling pathways.

3.9. The aqueous extract of PR protected pulmonary epithelial cells against PM2.5 exposure through activating AKT1 signalling pathway, and inhibiting STAT3 and JUN signalling pathways

To further explore the three key targets for the aqueous extract of PR in treating PM2.5-induced ALI, BEAS-2B cells were selected for *in vitro* experimental studies. Compared to the control group, treatment with 25–400 μ g/mL of PM2.5 significantly inhibited cell viability in a dose-dependent manner (Fig. 9A). BEAS-2B cells were pretreated with PR extracts, and then exposed to PM2.5 for 24 h, followed by the MTT assay. PM2.5 exposure group exhibited a significant reduction in cellular viability compared to the control group, whereas pretreatment with PR partially reversed this trend (Fig. 9B). The results of the inflammatory factor assay indicated that the levels of IL-6 and TNF- α were significantly elevated in the PM2.5 exposure group compared to the control group, while the release of IL-6 and TNF- α was inhibited following pretreatment with the extract of PR (Fig. 9C).

Next, intervention experiments were performed by using inhibitor or agonists of these signaling pathways. As illustrated in Fig. 9F–I, compared to the control group, PM2.5 treatment significantly inhibited p-AKT/AKT and enhanced p-STAT3/STAT3, whereas treatment with the extract of PR up-regulated p-AKT/AKT and down-regulated p-STAT3/STAT3. MK2206 or colivelin reversed the effect of PR extract on p-AKT/AKT and p-STAT3/STAT3. Furthermore, PR extracts inhibited PM2.5-induced high level of p-JUN, while this change was restored by anisomycin (Fig. 9D–F and H). Correspondingly, PR extract suppressed

Table 2

The dock binding energies of the bioactive compounds in PR and their targets.

Mol ID	Ingredients	Targets	PDB ID	Affinity/(kcal/mol)
MOL001689	Acacetin	TP53	3TG5	−9.8
MOL000006	Luteolin	TP53	3TG5	−9.8
		AKT1	4EJN	−9.6
		MAPK1	5WP1	−7.7
MOL006024	Deapio-platycodin D	STAT3	6NJS	−8.5
MOL006074	Platycodin D	JUN	1JNM	−8.6

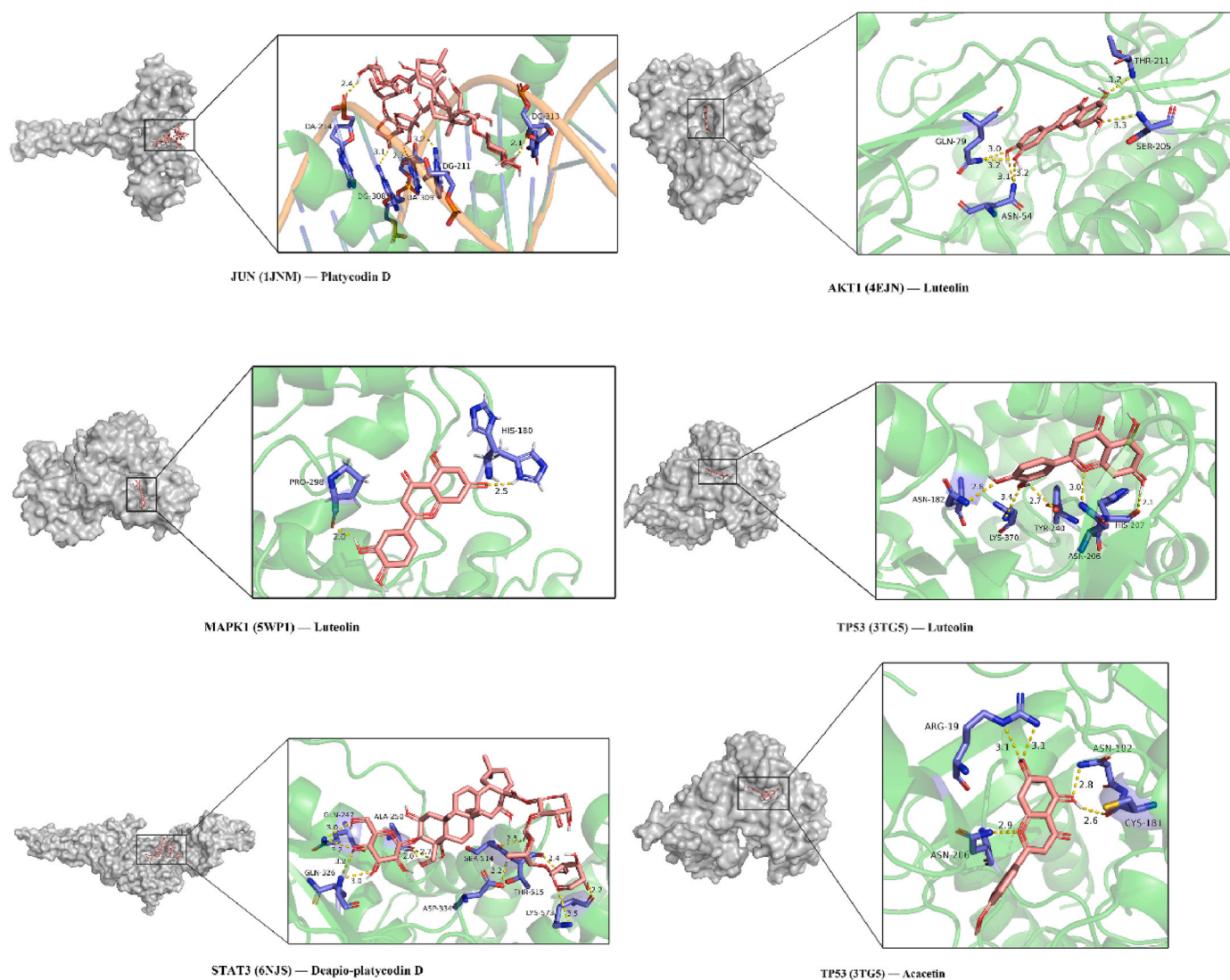


Fig. 4. The molecule docking of bioactive components of PR and their targets.

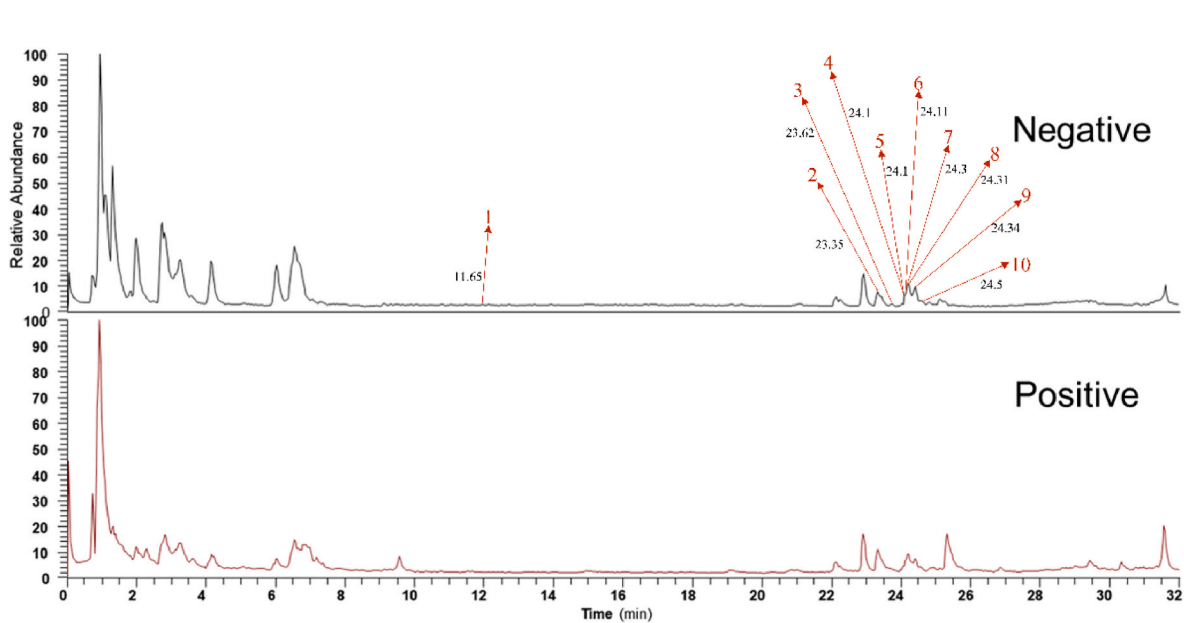


Fig. 5. Analysis of chemical components of PR extracts by LC-MS.

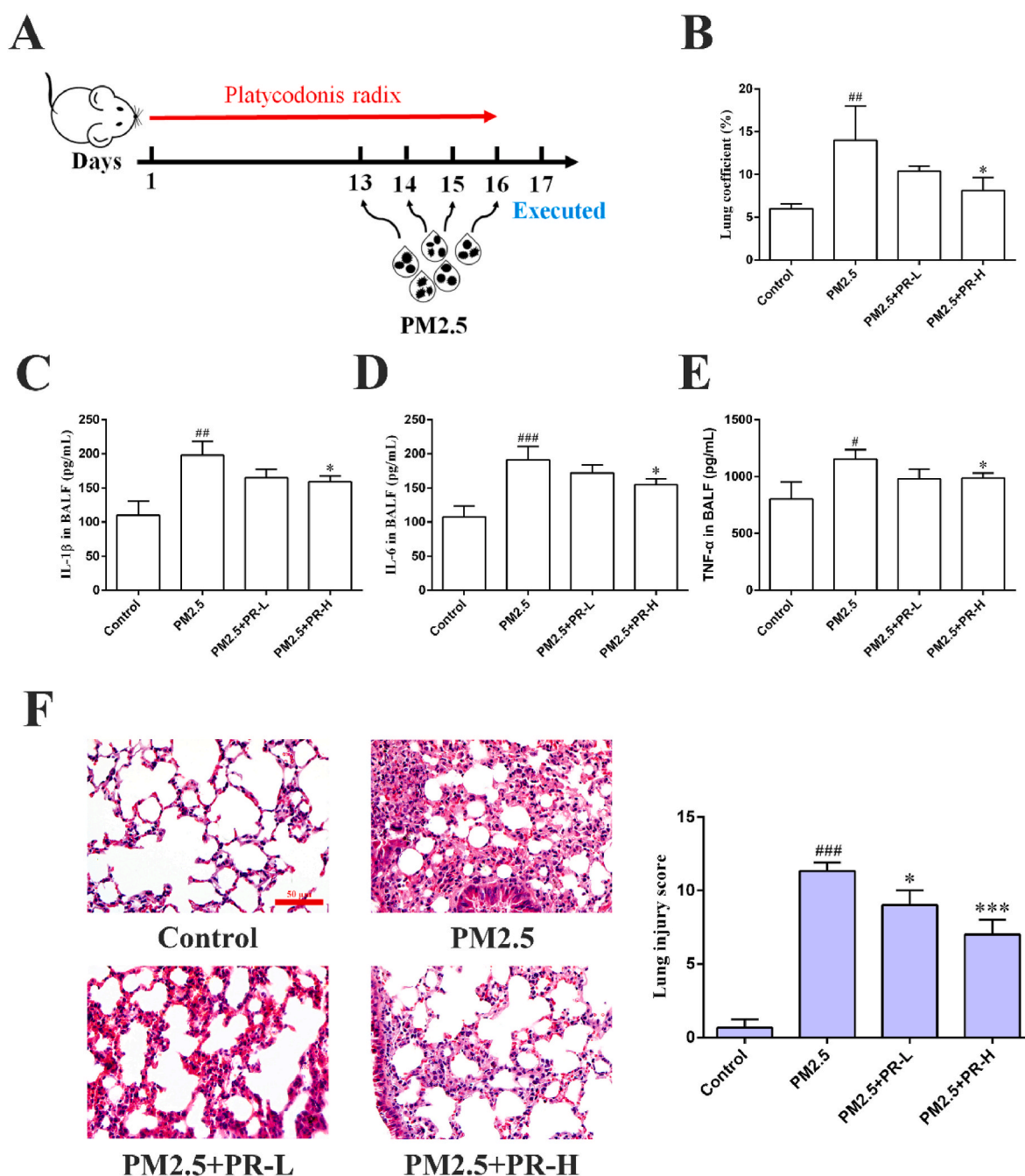


Fig. 6. Aqueous extract of PR improved PM2.5-induced ALI by decreasing the levels of inflammatory factors. (A) The model of PM2.5-induced ALI. (B) The lung coefficient of mice. The IL-1 β (C), IL-6 (D) and TNF- α (E) levels in the BALF of mice. (F) HE staining and injury score of lung tissue and lung injury score. [#]P < 0.05, ^{##}P < 0.01 and ^{###}P < 0.001 vs. control group, ^{*}P < 0.05 and ^{***}P < 0.001 vs PR group.

PM2.5-induced high level of IL-6 and TNF- α , whereas treatment with these inhibitor or activators reversed the effect of PR extracts ((Fig. 9E–G and I). Our results indicated that PR extract protected pulmonary epithelial cells against PM2.5 exposure through activating AKT signalling pathway, and inhibiting STAT3 and JUN signalling pathways.

4. Discussion

ALI is an oxidative stress response caused by air pollutants, micro-organisms, and chemicals that enter the human lungs. Among these pollutants, PM2.5 is particularly concerning, as it can provoke an acute pulmonary inflammatory response and cause lung damage. Exposure to high concentrations of PM2.5 results in cell membrane lysis,

mitochondrial ultrastructural damage, DNA damage, inflammatory response and oxidative stress in alveolar epithelial cells. PM2.5 can induce lung cell death through various mechanisms, including autophagy, apoptosis, pyroptosis and ferroptosis (Wang et al., 2021b). Furthermore, studies have shown that PM2.5 exposure can induce inflammatory response and lung injury in experimental animal models, such as mice and rats (Hou et al., 2024; Xie et al., 2022a).

PR, a common herb in the Korean Peninsula and northeastern China, has been widely used in the treatment of various inflammatory diseases. PR has been found to have the protective effect against ALI induced by LPS (Yang et al., 2023) and *Staphylococcus aureus* (Liu et al., 2019). This study aims to screen and analyze the active components of PR and potential core targets and pathways in treating ALI with network

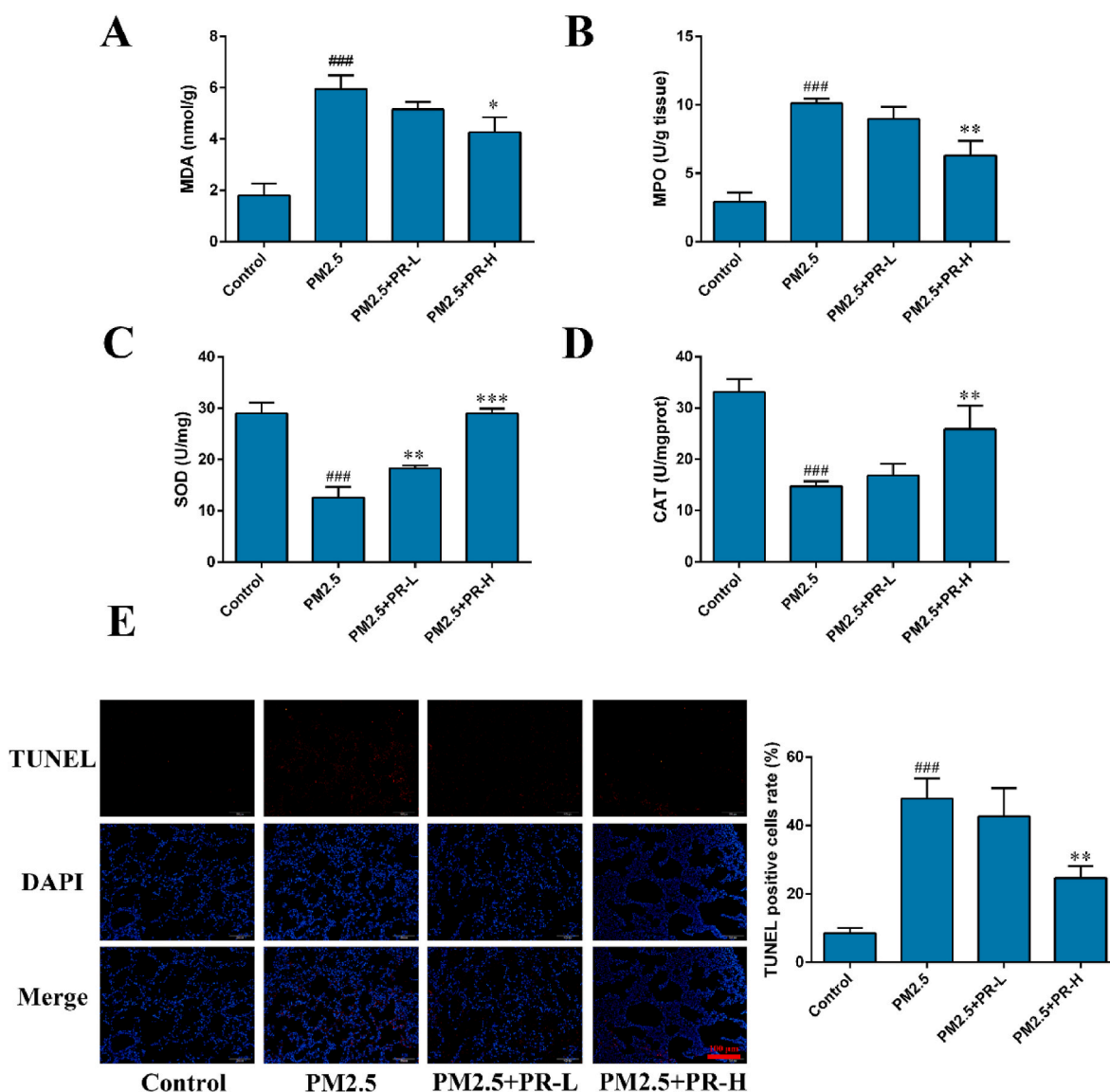


Fig. 7. Aqueous extract of PR ameliorated PM2.5-induced ALI by reducing oxidative stress and cell apoptosis. The MDA (A), MPO (B), SOD (C) and CAT (D) levels in the lung tissues of mice. (E) TUNEL assay in the lung tissues of mice and the corresponding quantitative analysis. ^{###}*P* < 0.001 vs. control group, ^{*}*P* < 0.05, ^{**}*P* < 0.01 and ^{***}*P* < 0.001 vs PR group.

pharmacology and molecular docking. Importantly, the protective effect of PR against PM2.5-induced ALI and the molecular mechanism were investigated by experiments *in vivo*. Our results showed that the aqueous extract of PR attenuated the pathological impairments to lung tissue, decreased the inflammatory factor levels in bronchoalveolar lavage fluid, enhanced the antioxidant capacity in lung tissue, and decreased lung cell apoptosis in mice exposed to PM2.5. Whereas, in the experiments *in vitro*, PR promoted the phosphorylation of AKT, inhibited the phosphorylation of STAT3 and c-JUN, and suppressed the expression of IL-6 and TNF- α , thus inhibiting the toxic effects of PM2.5 on BEAS-2B cells.

The outcomes of network pharmacology indicated that PR included mainly 16 bioactive components and eight of them, mainly platycodon saponins, could be detected in the aqueous extracts of PR by LC/MS assay. The bioactive components of PR and ALI shared a total of 156 targets. STAT3, AKT1, JUN and TP53 could be regarded as the core targets for the treatment of ALI by PPI analysis. The results of molecular docking confirm that there are strong intermolecular interactions between the active components and their targets, including acacetin and TP53, deapio-platycodin D and STAT3, and platycodin D and JUN.

Luteolin is also speculated to have strong interactions with TP53, AKT1 or MAPK1. Furthermore, LC/MS analysis showed that platycodin D and deapio-platycodin D were detected in the aqueous extract of PR, but no acacetin and luteolin were found, possibly due to their lipophilicity. Both platycodin D and deapio-platycodin D were reported to have strong anti-inflammatory activity and inhibit LPS-induced NO release in the RAW264.7 cells (Ahn et al., 2005). Platycodin D alleviated LPS-induced ALI by inhibiting apoptosis and inflammatory factor release (Tao et al., 2015). Platycodin D exerted its anti-ALI effect via the LXR α -ABCA1 signaling pathway (Hu et al., 2016). Activation of LXR α -ABCA1 signal could deplete cholesterol and prevent the transport of TLR4 to lipid rafts, leading to the destruction of lipid rafts. In addition, anti-inflammatory effect of platycodin D was also associated with inhibiting the NLRP3 inflammasome and the NF- κ B signaling pathway (Wu et al., 2021). Here, STAT3 and JUN had been considered as the main core targets of platycodin D and deapio-platycodin D by network pharmacology, respectively. A number of studies have shown that ALI can be alleviated by modulating the phosphorylation level of STAT3, JUN or AKT1 (Wang et al., 2020; Xie et al., 2022b; Zhao et al., 2016).

STAT3 plays an influential role in inflammation and is

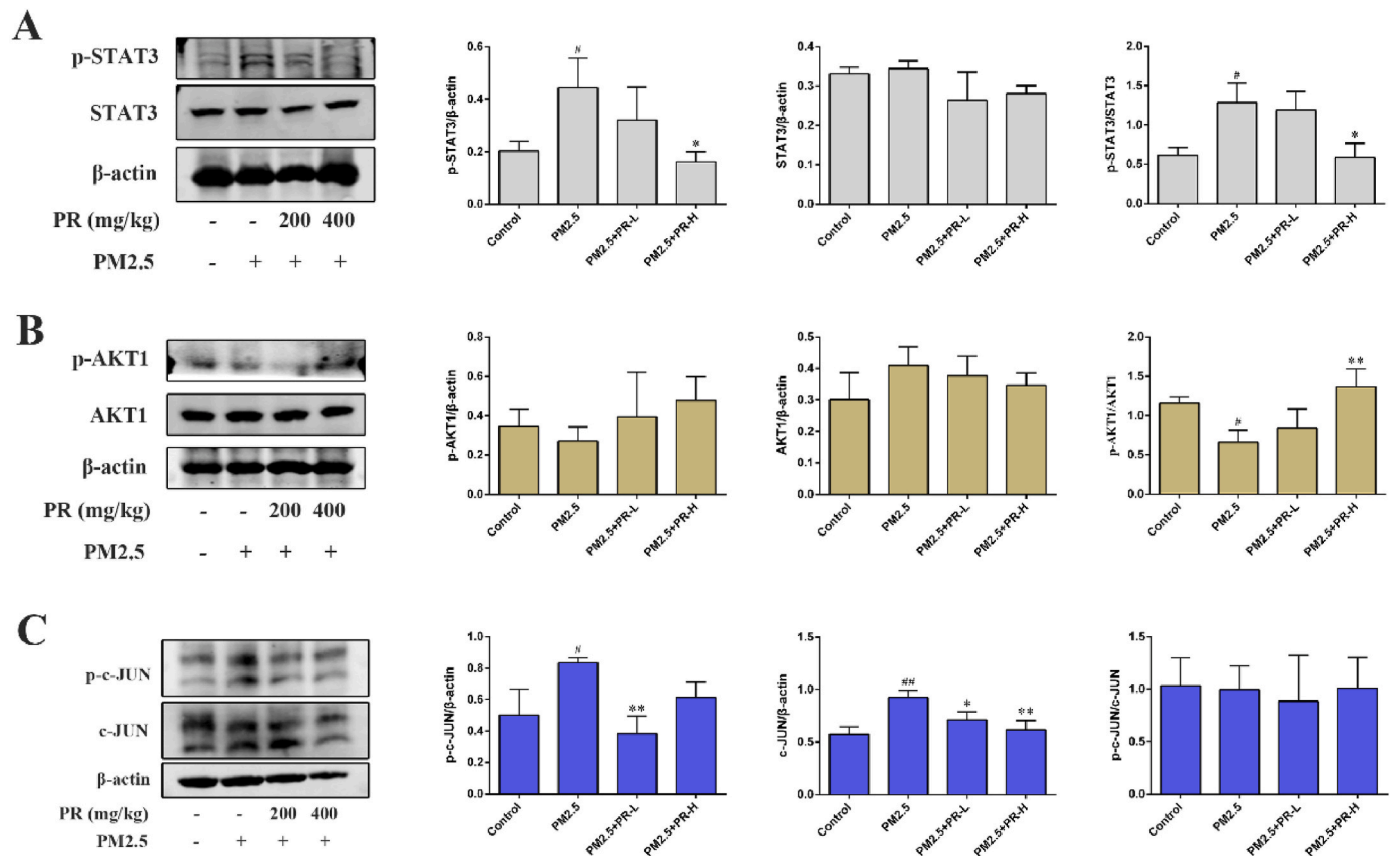


Fig. 8. Aqueous extract of PR alleviated PM2.5-induced ALI by modulating STAT3, AKT and JUN signaling pathways. The protein expression of p-STAT3/STAT3 (A), p-AKT1/AKT1 (B) and p-c-JUN/c-JUN (C) in the lung tissue of mice and the corresponding quantitative analysis. The data are expressed as mean $\pm$ SD of the results from three independent experiments. [#]P<0.05 and ^{##}P<0.01 vs. control group, ^{*}P<0.05 and ^{**}P<0.01 vs. PR group.

phosphorylated to increase the inflammatory response in response to tissue injury (Hillmer et al., 2016). Numerous studies have demonstrated that STAT3 is involved in the regulation of ALI. In the model of ALI induced by lipopolysaccharides (LPS) or bleomycin, etc., the phosphorylation level of STAT3 was significantly elevated (Peng et al., 2021; Wang et al., 2021a). Of note, STAT3 was also involved in PM2.5-induced ALI. The lung cell damage gradually increased with increasing concentrations of PM2.5, which was associated with the activation of STAT3/JNK pathways to regulate pro-inflammatory response (Jia et al., 2020). PM2.5 could also induce apoptosis in lung tissue cells by activating the JAK-2/STAT3 signaling pathway to disrupt the cell growth cycle (Yue et al., 2021). Here, *in vivo* experiments showed that the phosphorylation level of STAT3 significantly increased in the lung tissue of mice exposed to PM2.5, while the aqueous extract of PR inhibited the PM2.5-induced phosphorylation level of STAT3.

JUN (c-Jun) is one of the classical transcription factors in MAPK signaling pathway (Catalan et al., 2012). Both TNF- α and oxidative stress increase the binding and transcription activities of AP-1 (c-Fos/c-Jun) via the JNK signalling pathway (Rahman et al., 2002). In a LPS-induced ALI model, JNK activation led to a significant increase in the phosphorylation of c-Jun, thereby promoting the production of inflammatory factors (Xie et al., 2022b; Zhou et al., 2023). Previous studies indicate that the phosphorylated c-Jun can be translocated into the nucleus to activate the transcription of inflammatory factors, such as IL-8, TNF- α and IL-6 (Ip and Davis, 1998). Some active ingredients in foods, such as emodin and liquiritin, were reported to alleviate LPS-induced acute lung injury by inhibiting the phosphorylation of c-Jun (Xie et al., 2022b; Zhou et al., 2023). Similarly, in this study, PM2.5 up-regulated the phosphorylation level of c-Jun at Ser63 and promoted the release of inflammatory factors IL-1 β , IL-6, and TNF- α ,

whereas PR inhibited the phosphorylation of c-Jun and the secretion of these inflammatory factors, thereby alleviating acute lung injury. The phosphorylation of c-Jun on residues Ser63 and Ser73 induced by JNK can inhibit the ubiquitination and degradation of c-Jun, ultimately leading to the accumulation of c-Jun protein. Consistent with this situation, our results showed that PM2.5 could simultaneously up-regulate the phosphorylation level and total protein level of c-Jun, and these changes were reversed by the administration of PR extracts.

AKT1 is one of the targets analyzed by network pharmacology for PR treatment of ALI, but there are not many studies on its role in PM2.5-induced ALI. The PI3K/AKT pathway is one of the classic signaling pathways involved in the regulation of cell proliferation and apoptosis (Di Lorenzo et al., 2009), as well as in the regulation of inflammatory response (Ma et al., 2021). AKT1 also plays an essential role in cellular metabolism, glucose metabolism and gene expression. Studies have shown that PI3K/AKT plays a very important role in inflammation-mediated diseases, such as asthma and rheumatoid arthritis (Camps et al., 2005). Whether the PI3K/AKT pathway is activated or inhibited in ALI mainly depends on the inducing factors. Unlike other inducing factors, PM2.5-induced lung injury mainly leads to cell damage and various forms of cell death, including autophagy-induced apoptosis (Cao et al., 2024), pyroptosis (Huang et al., 2023a, 2023b) and ferroptosis (Wang et al., 2022b, 2023), which are also the main causes of PM2.5-induced lung injury. The PI3K/AKT signaling pathway plays an important role in maintaining cell membrane integrity (Lee et al., 2019), promoting cell proliferation (Di Lorenzo et al., 2009), inhibiting autophagy, and combating cell pyroptosis and ferroptosis. Therefore, abundant literature support that the p-AKT/AKT ratio in the lung tissue of mice exposed to PM2.5 was significantly reduced, while the increase in this ratio contributed to the recovery of lung damage.

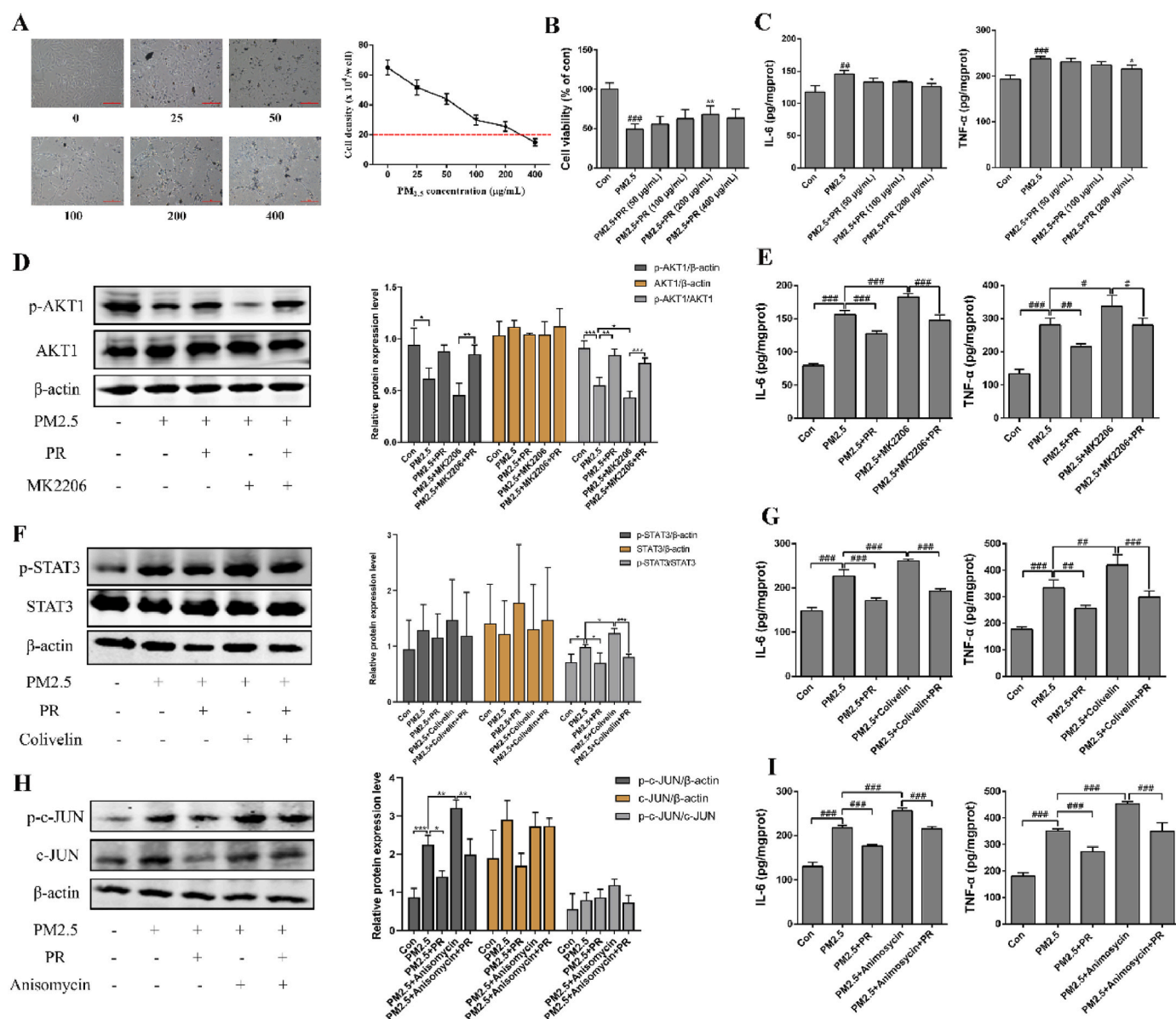


Fig. 9. Aqueous extract of PR protected pulmonary epithelial cells against PM_{2.5} exposure through activating AKT signalling pathway, and inhibiting STAT3 and JUN signalling pathways. (A) Morphological and number diagrams of BEAS-2B cells treated with 25–400 $\mu\text{g/mL}$ of PM_{2.5} for 24 h. (B) Effect of PR extract on the viability of BEAS-2B cells exposed to PM_{2.5}. (C) Effect of PR extract on the levels of IL-6 and TNF- α in BEAS-2B cells exposed to PM_{2.5}. BEAS-2B cells were pretreated with 200 $\mu\text{g/mL}$ of PR extract for 24 h, and then incubated with 50 μM of MK2206 (D), 12.5 $\mu\text{g/mL}$ of colivelin (F) or 4 μM of anisomycin (H), respectively, followed by exposure to 200 $\mu\text{g/mL}$ of PM_{2.5} for another 1 h. The protein samples were then collected for Western blot. The reversal effect of MK2206 (E), colivelin (G) or anisomycin (I) on the anti-inflammatory effect of PR in BEAS-2B cells exposed to PM_{2.5}. $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$ vs. control group, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ vs. PR group.

Wang et al. found that PM_{2.5} decreased the phosphorylation level of AKT1 to induce cell apoptosis in the lungs, while Ma xing shi gan decoction reversed this process to reduce PM_{2.5}-induced ALI (Wang et al., 2020). Huang et al. confirmed that PM_{2.5} induced lung cell pyroptosis by down-regulating the p-AKT/AKT signalling, while rosalidin activated PI3K/AKT pathway and prevented PM_{2.5}-induced pulmonary toxicity. Similar with these studies, our results also showed that the p-AKT/AKT ratio in the lung tissue of mice exposed to PM_{2.5} was significantly reduced. Compared with PM_{2.5} treatment group, the administration of high-dose PR extracts significantly up-regulated the expression level of p-AKT1/AKT1 and decreased cell apoptosis in lung tissue. Consequently, PR may alleviate ALI by up-regulating the AKT signaling and reducing lung cell apoptosis.

5. Conclusion

In summary, this study primarily analyzed the targets of PR therapy for ALI through network pharmacology, and validated them through LC-MS, molecular docking, and *in vivo* and *in vitro* experiments. PR inhibited the release of inflammatory factors, oxidative stress, and cell death in lung tissue of mice exposed to PM_{2.5} by regulating the phosphorylation levels of JUN, AKT1, and STAT3 signaling molecules, thus reducing pulmonary edema and alleviating PM_{2.5}-induced ALI. The role and mechanism of key components of PR in improving ALI will be studied in the subsequent work. Therefore, platycodonis radix has the possibility of being developed as a functional food or drug for alleviating lung injury caused by particulate matter.

CRediT authorship contribution statement

Xianlei Han: Writing – original draft, Validation, Data curation, Conceptualization. **Yue Zhang:** Visualization, Methodology, Investigation, Formal analysis. **Fan Zhang:** Writing – original draft, Visualization. **Xiumei Li:** Formal analysis, Conceptualization. **Yanli Meng:** Software, Methodology. **Jinhai Huo:** Software, Conceptualization. **Mian Chen:** Software, Formal analysis. **Fei Liu:** Methodology, Formal analysis. **Weiming Wang:** Methodology, Conceptualization. **Nan Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interests.

Data availability

I have shared the link to my data at the Attach File step

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2024.118829>.

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