

Unveiling the protective mechanism of pre- and post-processed Viticis Fructus against acute lung injury: Integrating network pharmacology, metabolomics, and experimental validation

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Keywords: Viticis Fructus, Processed Viticis Fructus, Acute lung injury, Network pharmacology, Metabolomics, PI3K/Akt pathway

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10952484/v1>

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Additional Declarations: No competing interests reported.

Abstract

Acute lung injury (ALI) causes high morbidity and mortality, with inflammation as its key pathogenic driver. *Viticis Fructus* (VF), an edible and medicinal plant traditionally used in East Asia, and its stir-fried processed form (PVF) possess anti-inflammatory activities, yet their molecular mechanisms against ALI are poorly understood. Herein, we combined LC-MS-based component identification, network pharmacology, metabolomics and experimental assays to clarify the anti-ALI mechanisms of VF and PVF. In LPS-challenged mice, VF and PVF mitigated lung pathological damage and suppressed inflammation and oxidative stress. Network pharmacology and molecular docking recognized core compounds (casticin, luteolin) and pivotal targets (STAT3, AKT1, IL-6) with favorable binding capacities. Multi-omics integration further verified that the PI3K-AKT pathway underpinned their protective actions. This study not only demonstrates the multi-component, multi-target therapeutic features of VF and PVF against ALI, but also highlights their potential as functional food ingredients for dietary prevention and adjuvant intervention of inflammatory lung diseases.

1. Introduction

There are several different causes of acute lung injury (ALI), which is a diffuse inflammatory lung injury. According to epidemiological data, 3 million individuals worldwide experience ALI annually, with a 40–60% fatality rate (Lihua et al., 2021). ALI has a complicated aetiology. The main risk factors include inflammatory cell infiltration into lung tissues and excessive production of pro-inflammatory cytokines (Yasmeen et al., 2016). Thus, the key to treating ALI is to reduce excessive inflammation (J et al., 2010). ALI is treated clinically using anti-inflammatory medications, mainly glucocorticoids (V. et al., 2022). Nevertheless, it has been demonstrated that glucocorticoids do not increase patient survival rates (Pathophysiology et al., 2019), and long-term use may result in major side effects (Albers et al., 2023). In this regard, exploring safe, natural anti-inflammatory bioactive components from edible and food-derived resources for the dietary prevention and adjuvant intervention of inflammatory lung diseases has become a research hotspot in food science and functional food development.

Viticis Fructus (VF), the dried ripe fruit of *Vitex trifolia* L. (VT) or *Vitex trifolia* L. var. *simplicifolia* Cham. (Y. Li et al., 2025), is a typical edible and medicinal plant widely distributed in East Asia (Y. Zhang et al., 2026). As both a traditional herbal medicine and a food ingredient, VF has been consumed in teas. In traditional Chinese medicine, VF is ascribed to the lung, liver, and bladder meridians and possesses the functions of dispersing wind-heat, clearing and benefiting the head and eyes, and relieving arthralgia (Tian et al., 2024). VF is rich in flavonoids, terpenoids and phenolic acids, and previous food chemistry and nutritional studies have confirmed its multiple functional activities including anti-inflammation (Z. Zhang, W. Li, et al., 2026), antioxidant and immunomodulatory effects (Z. Zhang, K. Zhang, et al., 2026). Stir-frying is a traditional processing technology commonly applied to edible herbal materials, which can not only improve the flavor and sensory properties, but also regulate the composition of bioactive components and enhance specific functional activities—an aspect of direct relevance to food processing and product development. Extensive pharmacological studies have shown that VF significantly alleviates

inflammation (DucDat et al., 2023), diabetes (A & A.H., 2016), and cardiovascular diseases both before and after processing (Oh et al., 2024). According to earlier studies, VF possesses anti-inflammatory qualities that are attained via blocking the mitogen-activated protein kinase (MAPK) signaling pathway and inflammatory cytokines (Choudhary et al., 2025). Furthermore, in a mouse model, casticin, an active substance extracted from VF, reduces acute lung inflammation brought on by cigarette smoke (Lee et al., 2015). However, the exact mechanisms underlying the effects of VF and processed VF (PVF) on ALI remain largely unknown, particularly regarding how thermal processing alters their bioactive profile and pharmacological efficacy.

Through a variety of protein factors, the PI3K/Akt pathway, a crucial intracellular signaling system (W. Li et al., 2025), controls cell differentiation, proliferation, activation, and antiviral responses (Xingxu et al., 2023). Lung inflammation is adversely regulated by the PI3K/Akt signaling pathway, according to a number of recent in vitro and in vivo investigations. This pathway provides negative feedback regulation of inflammatory responses, representing a novel therapeutic target for lung injury diseases (Luo et al., 2025). We hypothesized that VF and PVF prevent ALI by inhibiting inflammasome activation through regulating the PI3K/Akt pathway.

In order to thoroughly investigate and validate drug-disease interactions, integrative pharmacology—a new interdisciplinary approach—combines chemical composition analysis, pharmacodynamics, network pharmacology, multi-omics studies, and molecular docking (Xun et al., 2021). Therefore, when combined with TCM philosophy, integrative pharmacology can successfully discover the active components and mechanisms of TCM in treating ALI, with a particular focus on how processing-induced chemical changes contribute to enhanced anti-inflammatory activity—providing a scientific basis for developing VF-based functional foods. LPS was used in this investigation to produce an ALI model. The lung-protective effects and mechanisms of VF and PVF were then examined using an integrated pharmacological analytic approach.

2. Materials and methods

2.1 Reagents

Beijing Chemical Plant (Beijing, China) provided n-butanol and ethyl acetate. Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) supplied the formic acid. Chromatographic methanol was acquired from Thermo Fisher Scientific, Inc. (Shanghai, China). LPS (*Escherichia coli* 055: B5) and deuterium oxide (99.9%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies (P-PI3K, P-Akt, CD44, β -actin, and CD133) were purchased from Affinity Biosciences (Jiangsu, China). Enzyme-linked immunosorbent assay (ELISA) kits for detecting TNF- α , PKB α , and IL-6 were obtained from Jiancheng Institute of Bioengineering (Nanjing, China).

2.2 Preparation of drug administration samples of VF and PVF

VF (100 g) was obtained from Anqing, Anhui, China (batch number: 240411). A voucher specimen was deposited at the School of Pharmacy, Jiangsu Ocean University, Lianyungang, China. VF was stir-fried at 140 °C for 5 min, cooled completely, ground, and sieved (40 mesh). VF and PVF powders (50.0 g) were weighed separately, soaked in methanol (10 times the powder weight) at room temperature for 30 min, and reflux-extracted at 65 °C for 1 h. The filtrates were combined, concentrated under reduced pressure to 1.0 g/mL, freeze-dried to remove residual methanol, and stored at -20 °C. Before administration, samples were thawed and prepared with a 1% carboxymethyl cellulose sodium (CMC Na) solution.

2.3 Chemical analysis of VF and PVF

UPLC analysis was performed using an Ultimate3000 system (Thermo Fisher Scientific, USA). Chromatographic separation was conducted on a Hypersil GOLD aQ-C18 column (100 × 2.1 mm, 1.9 µm). The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B), with the following gradient elution program: 95–95% B (0–2 min); 95 – 5% B (2–42 min); 5–5% B (42–47 min); 5–95% B (47–47.1 min); 95–95% B (47.1–50 min). The flow rate was 0.3 mL/min, and the column temperature was 40°C.

MS conditions were optimized as follows: ion source temperature of 500 °C (positive mode) and 450 °C (negative mode); curtain gas flow rate of 50 L/min; nebulization and auxiliary gas flow rates of 50 L/min; spray voltage of 5500 V (positive mode) and 4400 V (negative mode). MS and MS/MS scanning ranges were m/z 100–1200 and m/z 50–1000, respectively.

2.4 Network pharmacology

2.4.1 Screening of active compounds and targets of VF

Based on LC-MS analysis results, known compounds of VF were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP, <http://tcmsp.w.com/tcmsp.php>) database. A review of the literature revealed more active substances. 43 active compounds in all were chosen for additional examination.

The Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>) and the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) were used to gather potential gene targets of VF. Potential targets were chosen at a probability score of ≥ 0.1 .

2.4.2 Screening of ALI targets

ALI-associated target genes were identified using the keywords “pneumonia” and “inflammation of the lungs”. A total of 1979 genes related to ALI were retrieved from the Online Mendelian Inheritance in Man (OMIM; <https://omim.org/>) and GeneCards (<https://www.genecards.org/>) databases. Common targets of VF and ALI were identified using the VennDiagram package, and a Venn diagram was generated.

2.4.3 Enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) analysis were carried out using the DAVID database (<https://david.ncifcrf.gov/>), with statistical significance set at $p < 0.05$.

2.4.4 Molecular docking

Based on the component-target and protein-protein interaction (PPI) network analyses, active components of VF and targets with high degree values were selected. Component structures in SDF format were obtained from PubChem, and target ligand structures were retrieved from the Protein Data Bank (PDB, <https://www.rcsb.org/>). The 3D structures were imported into the Molecular Operating Environment (MOE) software for molecular docking analysis.

2.5 Animals and treatments

Balb/c rats (20 ± 2 g) were purchased from SPF Biotech (Suzhou) Co., Ltd. [SCXK (Suzhou) 022 – 0006]. All animal procedures were approved by the Ethics Committee of SPF Biotech (Suzhou) Co., Ltd. (approval no. NAALH-W-2411035) and conducted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Rats were housed at controlled temperature (22 ± 2 °C), humidity ($50 \pm 5\%$), and under a standard 12 h light/dark cycle, with free access to food and water.

Balb/c rats ($n = 36$) were randomly divided into six groups ($n = 6$ per group): blank, control (NS), model (LPS, 2 mg/kg), LPS + VF (4.55 g/kg), LPS + PVF (4.55 g/kg), and LPS + dexamethasone (DEX, 2 mg/kg). VF, PVF, and DEX groups received their respective treatments by gavage daily for 7 days. Control and model groups were administered saline. On day 7, all groups except the control received intratracheal LPS instillation. The final treatment was administered 24 h after LPS instillation. Rats were sacrificed 2 h after VF and PVF administration, and bronchoalveolar lavage fluid (BALF), blood, and lung tissues were collected.

2.6 Lung wet/dry weight ratio (W/D)

After the upper-left lung tissue's surface moisture was eliminated, the wet weight was determined. After that, the tissue was dried for 48 hours at 80°C in a constant-temperature oven. Following drying, the W/D ratio was computed and the dry weight was measured.

2.7 Histological examination

Lung tissues were embedded in paraffin, fixed in 4% paraformaldehyde, gradually dehydrated with ethanol, and stained with hematoxylin and eosin (H&E). An optical microscope with a 400× magnification was used to observe pathological alterations, and pictures were taken.

2.8 Myeloperoxide (MPO) activity

MPO activity in lung tissue was measured using an MPO assay kit (Jiancheng Bioengineering Institute, Nanjing, China) at 450 nm.

2.9 Measurement of TNF- α , IL-1 β , and IL-6 levels

BALF was centrifuged for 10 minutes at 1500 rpm and 4°C to pellet the cells, which were then resuspended in 100 µl PBS. The ELISA kit's instructions were meticulously followed in determining the amounts of TNF-α, IL-6, and PKBα in the serum and BALF supernatant.

2.10 Metabolic profiling by ultraperformance liquid chromatography/mass spectrometry (LC-MS)

Lung tissue was thawed and homogenized in 2 mL methanol solution, then centrifuged (12,000 rpm, 4°C) for 10 min. The supernatant was collected, transferred to a 2 mL centrifuge tube, concentrated, and dried. It was then accurately reconstituted in 150 µL of 2-chloro-L-phenylalanine solution (80% methanol-water). The solution was filtered through a 0.22 µm membrane, and the filtrate was transferred into a detection vial for LC-MS analysis.

The analysis was conducted using a tandem mass spectrometry system (UPLC: Vanquish; MS/MS: Orbitrap Exploris 120) coupled with ultra-performance liquid chromatography. Chromatographic conditions included a Waters ACQUITY UPLC® HSS T3 column (2.1 × 100 mm, 1.8 µm) and a solvent system composed of water (0.1% formic acid) and acetonitrile (0.1% formic acid). The elution gradient (water/acetonitrile, v/v) was set as follows: 0 min (100:0), 1 min (92:8), 8 min (2:98), 10 min (2:98), 10.1 min (92:8), and 12 min (92:8). The flow rate was 0.3 mL/min, column temperature was 40°C, and injection volume was 2 µL. The effluent was connected to an electrospray ionization (ESI) triple quadrupole linear ion trap. The ESI parameters included: temperature 325°C, ion spray voltage 3500 V, ion source gas I 40 arb, and gas II 10 arb.

2.11 Western blot

Total proteins from lung tissues were extracted using RIPA lysis buffer. Protein concentration was measured using a BCA Protein Assay Kit (KGP902). Equal quantities of proteins were separated by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). Membranes were incubated overnight at 4°C with primary antibodies: mouse anti-p-AKT (1:200, 66444-1-Ig, Proteintech Group, China), rabbit anti-p-PI3K (1:2000; ab182651, Abcam Trading, China), mouse anti-CD44 (1:200, 60224-1-Ig, Proteintech Group, China), and rabbit anti-CD133 (1:200, 18470-1-AP, Proteintech Group, China). Following incubation, membranes were incubated with secondary antibodies. Blue Plus® II Protein Marker (DM111-02, TransGen Biotech, China) was used. Protein bands were analyzed using ImageJ software and normalized to the loading control.

2.12 Immunofluorescence staining

Lung tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval using 10 mM sodium citrate buffer (pH 6.0) heated in a microwave for 10 min. After cooling to room temperature and rinsing with PBS, the sections were incubated with primary antibodies and secondary antibodies, and then counterstained with DAPI for nuclear staining. Following dehydration and fixation, images were acquired using a digital whole-slide imaging scanner (Olympus).

2.14 Statistical analysis

The mean $\pm$ standard deviation (SD) is used to express data. Student's t-test was used to examine statistical differences between two groups, with a significance level of $p < 0.05$. SIMCA 11.0 software (Umetrics, Umea, Sweden) was used to conduct principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA). GraphPad Prism 5.0 was used to quantify and statistically analyze the data.

3. Results

3.1 Identification of active components in VF and PVF

Upon comparing mass spectrometry (MS) data in positive and negative ion modes, certain compounds were detected only in one of the two ion modes. Thus, data were collected using both modes. Based on chromatographic behavior, MS fragmentation patterns, previously reported data, and the TCMSP database, a total of 78 compounds in VF and 77 compounds in PVF were tentatively or definitively identified. These mainly included phenolic acids, flavonoids, terpenoids, and other components. The base peak chromatograms for VF and PVF extracts, in both positive and negative ion modes, are presented in Fig. 1. Retention times and MS data are detailed in Table S1.

Figure 1 Total ion chromatograms (TICs) for VF (A: positive ion mode; B: negative ion mode) and PVF (A: positive ion mode; B: negative ion mode)

3.2 Network pharmacology predicts potential mechanisms of VF and PVF treatment in ALI

57 medications and 648 illness targets were found in pertinent databases in order to investigate the molecular mechanisms of VF therapy in ALI. 175 putative VF protein targets associated with ALI therapy were identified using intersection analysis (Fig. 2A). Figure 2C shows the network of possible targets. These targets were linked to biological processes like inflammatory response, lipopolysaccharide response, and positive control of cytokine production, according to a subsequent GO analysis (Fig. 2D). Membrane rafts, vesicle lumens, and membrane microdomains were the key locations where cellular components were enriched. Endopeptidase activity, serine hydrolase activity, and serine-type peptidase activity were examples of molecular functions. The PI3K-Akt, HIF-1, and TNF signaling pathways were significantly enriched, according to KEGG pathway analysis (Fig. 2E).

3.3 Molecular docking

Three core targets (STAT3, AKT1, and IL6), identified from the PPI degree ranking, were selected for molecular docking based on network pharmacology analysis. These targets were docked with the top-ranked compounds (vitexin and luteolin) identified from the component-target network. Representative docking results are shown in Fig. 2G. The stability of receptor-ligand binding depends on the binding energy, with lower energies indicating more stable binding conformations. The results indicated that the targets exhibited favorable binding scores with the selected components.

Figure 2 Prediction of the potential mechanism of VF and PVF treatment of ALI. (A) Venn diagram of drug active components and disease targets. (B) PPI network. The color depth represents p-value, and the darker the red, the more significant it is. (C) Network analysis of compound-target interactions, yellow nodes represent traditional Chinese medicine, red nodes represent compounds, blue nodes represent targets, and purple nodes represent signal pathways. (D) GO enrichment analysis of VF. The color depth represents p-value, and the darker the red, the more significant it is. (E) KEGG pathway analysis of potential treatment targets of VF. The color depth represents p-value, and the darker the red, the more significant it is. (F) Molecular docking diagrams. (a) 3D molecular docking of Casticin and STAT3; (b) Luteolin and STAT3; (c) Casticin and AKT1; (d) Luteolin and AKT1; (e) Casticin and IL6; (f) Luteolin and IL6

3.4 Protective effects of VF and PVF against LPS-induced ALI in rat

To investigate the therapeutic effects of VF and PVF, ALI-induced rats were treated with these substances. After that, lung tissues were stained with H&E. As anticipated, the LPS-challenged group showed notable histological changes in comparison to the control group, such as thickening of the alveolar wall, interstitial edema, and widespread infiltration of inflammatory cells into alveolar spaces. Treatment with DEX, VF, and PVF reduced these pathological alterations (Fig. 3A).

MPO activity in BALF and the lung wet/dry (W/D) ratio are important markers of microvascular permeability and pulmonary edema. The W/D ratio (Fig. 3B) and MPO activity (Fig. 3C) significantly increased after LPS treatment ($p < 0.0001$). However, these parameters decreased in VF and PVF groups, similar to the effects observed with DEX treatment. These results indicate that VF and PVF significantly protect against LPS-induced disruption of the alveolar-vascular barrier, lung edema, cellular damage, and oxidative stress.

3.5 VF and PVF inhibit inflammatory responses in LPS-induced ALI

Local production of pro-inflammatory cytokines (IL-6, TNF- α , and PKB α) is crucial for neutrophil infiltration during ALI progression. Thus, the ability of VF and PVF to downregulate these cytokines was investigated. Cytokine levels in serum and BALF were quantified using ELISA (Fig. 3D-I). VF and PVF treatment significantly inhibited the LPS-mediated upregulation of IL-6, TNF- α , and PKB α . These findings correlate well with histopathological improvements and reduced microvascular permeability observed after VF and PVF treatment. The results suggest VF and PVF exert significant anti-inflammatory effects in ALI.

Figure 3 Protective effects of VF and PVF against LPS-induced ALI in vivo. (A) Lung tissue sections stained with H&E for histopathological analysis (magnification: 400 $\times$, $n = 6$). (B) MPO activity in lung tissue. (C) Lung wet/dry ratio; VF and PVF inhibit inflammatory responses in LPS-induced ALI in mice. (D-

l) Levels of inflammatory cytokines (IL-6, TNF- α , PKBa) in serum and lung tissues. Data are expressed as mean $\pm$ SD. *** $p < 0.001$, **** $p < 0.0001$ vs. CON group; ## $p < 0.01$, #### $p < 0.0001$ vs. MOD group

3.6 Metabonomics analysis of ALI rats treated with VF and PVF

3.6.1 Metabolite profile analysis

To reveal the mechanisms underlying VF and PVF treatment of ALI, lung tissues were analyzed using the UPLC-Orbitrap Exploris 120-MS method (Fig. 4A-B). A total of 1975 metabolites were identified in the VF group, and 2033 metabolites in the PVF group, primarily including flavonoids, terpenoids, and alkaloids (Fig. 4C).

Figure 4 TICs of VF (A) and PVF (B). (C) Classification of metabolites identified in mouse lung tissue

3.6.2 Multivariate analysis

To explore metabolite changes in ALI rats before and after treatment, OPLS-DA was conducted. The OPLS-DA score plot showed distinct separation among the control, model, and treatment groups, with the VF and PVF groups closely approaching the control group (Fig. 5A, B). These findings indicate that VF and PVF improved metabolic disturbances caused by ALI. To investigate differential metabolites between VF and PVF, volcano plot analysis was conducted (Fig. 5C). Compared with the VF group, the PVF group showed 444 significantly differential metabolites ($p < 0.05$, $VIP > 1$). These differences may result from compositional changes in VF after stir-frying, causing alterations in mouse metabolic processes, leading to significant up- or down-regulation of metabolites. Detailed information on these metabolites is provided in Table S2.

Figure 5 Metabolomic characteristics and differential metabolite analysis of mouse lung tissue ($n = 3$). (A-B) OPLS-DA score plots showing metabolic profiles of the CON, MOD, VF, and PVF groups. (C) Volcano plot of metabolite changes

3.6.3 Metabolic pathways analysis

To further examine the significance of differential metabolites, metabolic pathways were analyzed using MetaboAnalyst (www.metaboanalyst.ca). Based on $-\log(P)$ and count values, potential pathways involved in PVF treatment of ALI were identified and presented in a bubble plot (Fig. 6). These pathways primarily included: (a) biosynthesis of phenylpropanoids, (b) propanoate metabolism, (c) cysteine and methionine metabolism, (d) biosynthesis of alkaloids derived from ornithine, lysine, and nicotinic acid, and (e) reductive carboxylate cycle (CO_2 fixation).

Figure 6 KEGG pathways identified by metabolomics

3.7 VF and PVF exert therapeutic effects on ALI by modulating the PI3K/Akt signaling pathway

Western blot examination revealed that the model group's expression of the PI3K/Akt signaling components (P-PI3K and P-AKT) was significantly lower than that of the control group. Treatment with the positive control (DEX), as well as VF and PVF, effectively reversed this decline. The DEX, VF, and PVF groups had significantly greater P-PI3K and P-AKT ratios than the model group ($p < 0.05$), as shown in Fig. 7A-F. Furthermore, the model group's CD44 protein expression was much higher than the control group's, but it dropped once the medication was administered. On the other hand, the model group had higher levels of CD133, a marker for stem cell repair, which increased even more following medication delivery. These results suggest that PVF attenuates LPS-induced ALI more successfully than VF. Western blot results show that VF and PVF have anti-inflammatory effects by controlling the PI3K/Akt signaling cascade, which is essential for inflammatory responses.

Immunofluorescence staining further confirmed that VF and PVF exert therapeutic effects on ALI through the PI3K/Akt pathway (Fig. 7G). These immunofluorescence results aligned with the findings from the Western blot analysis, showing a consistent positive correlation in expression levels of P-PI3K, P-AKT, CD44, and CD133. Moreover, these findings confirmed that PVF had a stronger therapeutic effect compared to VF.

4. Discussion

ALI is a severe inflammatory disease characterized by poor prognosis and difficulty in treatment (Yasmeen et al., 2016). The high morbidity and mortality rates associated with ALI impose significant burdens on patients, families, and society. Despite over two decades of research, no breakthroughs have been achieved, and the adverse effects of current anti-inflammatory drugs limit their clinical applications (Albers et al., 2023). Thus, developing safer and more effective anti-inflammatory medications is urgently required. VF, a traditional edible medicinal plant (Y. Zhang et al., 2026), exhibits notable anti-inflammatory activity. Given its long history of culinary use and low toxicity, VF represents a promising candidate for functional food development aimed at respiratory health. Therefore, we hypothesized that VF may have therapeutic potential for ALI and investigated its therapeutic effects and underlying mechanisms.

Interleukin-6 (IL-6) is a critical cytokine in inflammatory responses (Musharaf & Nashwan, 2025). When the body experiences infection or tissue injury, inflammatory cells rapidly produce IL-6, playing a pivotal role in pneumonia development (Akgun et al., 2025). Research has shown that LPS-induced ALI increases the release of inflammatory mediators such as IL-6 and tumor necrosis factor- α (TNF- α) (Chen et al., 2025), and that lung inflammation can be reduced by inhibiting these mediators (Ma et al., 2025; Su et al., 2024). Additionally, blocking inflammatory cytokines including TNF- α and IL-6 reduces lung inflammation in *Mycoplasma pneumoniae* pneumonia in rats (Wang et al., 2024) by downregulating the NF- κ B signaling pathway (Feng et al., 2021). Pharmacodynamic results showed that VF and PVF significantly alleviated LPS-induced histopathological changes, including interstitial edema, inflammatory cell infiltration into alveolar spaces, and increased vascular permeability. Myeloperoxidase (MPO) serves as a biomarker to indirectly reflect neutrophil infiltration into inflamed tissues. VF and PVF protected against LPS-induced ALI by inhibiting MPO activation in vivo. In the LPS-induced ALI model, IL-6 and TNF- α levels were significantly upregulated, while PKB α was significantly downregulated.

Consistent with previous studies, VF and PVF treatment significantly improved MPO activity and reduced TNF- α , IL-6, and PKBa levels in serum and BALF, confirming their protective effects against LPS-induced ALI.

Network pharmacology explains drug effects on biological network disruptions through a holistic regulatory perspective, offering novel research approaches and methods for exploring the mechanisms of action of CMM and TCM formulations (Xin et al., 2021). By building and displaying drug-target-disease interaction networks, it enables thorough analysis of multi-component pharmacological mechanisms. The findings suggested that the main active ingredients of VF and PVF in the prevention and treatment of ALI might include berberine, vitexin, and luteolin. Significant enrichment in inflammatory signaling pathways, including the JAK/STAT3, PI3K/AKT, and TLR4/NF- κ B pathways, was found by KEGG signaling pathway enrichment analysis, which is consistent with their documented anti-inflammatory properties. This study then examined lung tissue metabolites in rats with ALI using metabolomics analysis. Notably, stir-frying processing (PVF) consistently showed superior protective effects compared with raw VF, as evidenced by lower inflammatory cytokines, improved histopathology, and enhanced modulation of the PI3K/Akt pathway. This processing-induced potentiation is likely attributable to the thermal conversion of certain glycosides to aglycones or the generation of new bioactive compounds, as observed in our chemical profiling (Table S1). From a food science perspective, such thermal treatment mimics common culinary practices (e.g., roasting, stir-frying) that can alter the phytochemical composition and bioactivity of plant-based ingredients. Therefore, our findings underscore the importance of processing parameters in optimizing the functional properties of VF for potential use as a dietary supplement or functional food ingredient.

Metabolomics provides an effective approach for observing dynamic changes of small-molecule metabolites in vivo (Department of Analytical Chemistry et al., 2019). A major pathogenic component of Gram-negative bacteria's cell walls, lipopolysaccharide (LPS) frequently causes inflammation. The production and release of inflammatory mediators (Almeida et al., 2025), such as COX-2, inducible nitric oxide synthase (iNOS) (Choudhary et al., 2025), and pro-inflammatory cytokines including TNF- α and IL-6 (Akgun et al., 2025). In order to investigate changes in lung tissue metabolites, an intratracheal injection of LPS was used to create a mouse pneumonia model. The citrate cycle, propanoate metabolism, and reductive carboxylic acid cycle were the main mechanisms by which VF and PVF prevented LPS-induced pneumonia, according to KEGG enrichment analysis of differential metabolites. The pathogenesis and progression of pneumonia closely associate with inflammation, and these pathways play central roles. These results suggest that VF and PVF effectively alleviate LPS-induced pneumonia in mice by modulating these metabolic pathways.

Inflammatory reactions depend on the PI3K/Akt signaling pathway (Xingxu et al., 2023). By reducing the release of pro-inflammatory cytokines like TNF- α and IL-6, activation of this system lowers inflammation. Additionally, PI3K/Akt reduces CD44 expression by negatively regulating NF- κ B (Feng et al., 2024). By suppressing immune cell activation and cytokine release, the PI3K/Akt pathway exhibits substantial anti-inflammatory effects. Additionally, CD133 is a marker of stem cell-mediated repair, and improvement in

this indicator contributes to ALI therapy. In this study, protein expression and fluorescence intensities of p-PI3K, p-Akt, CD44, and CD133 showed corresponding increases or decreases. Protein expression analysis demonstrated that VF and PVF alleviated the inflammatory response in ALI by activating the PI3K/Akt pathway (Fig. 8). Collectively, our integrative approach not only deciphers the molecular network by which VF and PVF alleviate ALI, but also provides a practical paradigm for evaluating the health benefits of edible medicinal plants in the context of food science.

5. Conclusion

This study, through an integrative pharmacology strategy, comprehensively characterized the anti-ALI mechanisms of raw and processed VF, and demonstrated that stir-frying processing significantly enhances its therapeutic efficacy. Our systems-pharmacology approach revealed that VF and PVF ameliorate ALI by activating the PI3K/Akt signaling pathway, reducing inflammatory cytokines, and modulating energy metabolism. Beyond the mechanistic insights, these findings highlight the potential of VF—particularly its processed form—as a functional food ingredient or dietary supplement for the prevention and adjuvant management of inflammatory lung diseases. The processing-induced bioactivity enhancement also provides a scientific rationale for optimizing traditional food-processing techniques to improve the health-promoting properties of edible botanicals. Furthermore, the integrative analytical framework established here offers a versatile reference for future investigations into the functional mechanisms of complex plant-based food systems.

Abbreviations

ALI	acute lung injury
VF	Viticis Fructus
TCM	traditional Chinese medicine
VT	Vitex trifolia L.
VTS	Vitex trifolia L. var. simplicifolia Cham.
MAPK	mitogen-activated protein kinase
LPS	lipopolysaccharide
ELISA	enzyme-linked immunosorbent assay
CMC Na	carboxymethyl cellulose sodium

DEX
dexamethasone
BALF
bronchoalveolar lavage fluid
W/D
lung wet/dry weight ratio
H&E
hematoxylin and eosin
MPO
myeloperoxide
TCMSP
the Traditional Chinese Medicine Systems Pharmacology
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
PPI
protein-protein interaction
PDB
the Protein Data Bank
MOE
the Molecular Operating Environment
LC-MS
Metabolic profiling by ultraperformance liquid chromatography/mass spectrometry
ESI
electrospray ionization
SD
standard deviation
PCA
principal component analysis
OPLS-DA
orthogonal partial least-squares discriminant analysis
MS
mass spectrometry
IL-6
interleukin-6
TNF- α
tumor necrosis factor- α
Inos
inducible nitric oxide synthase.

Declarations

Funding sources

This work was supported by Lianyungang Huaguoshan Talent Program - Double Innovation Talent Project (2024), Jiangsu Key Laboratory on Innovation for Marine Medicine and Modern Chinese Medicine (BM2024008) and the National Nature Science Foundation of China (82404860, 82374038).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Y. Zhang. and Y. Li. wrote the main manuscript text, G. Xun. prepared figures 1-2, X. Zong. prepared figures 3-4, C. Zhang. prepared figures 5-6, Y. Shi. prepared figures 7-8. and K. Qin. provided funding. All authors reviewed the manuscript.

References

1. A, S. M., & A.H., D. (2016). Hypoglycemic and hypolipidemic effects of ethyl acetate leaf extract of *Vitex simplicifolia* in alloxan induced diabetic wister rats. *Bayero Journal of Pure and Applied Sciences*, 9(1), 223. <https://doi.org/10.4314/bajopas.V9i1.35>
2. Akgun, Y., Baykara, Y., & Pepkowitz, S. H. (2025). Interleukin 6 inhibition may be effective in mitigating the severity of transfusion-related acute lung injury. *Rhode Island Medical Journal* (2013), 108(1), 49–50. https://kns.cnki.net/kcms2/article/abstract?v=tC3tAMdiXNcVsyPtXvZNMQd-tqTj8PruEE9oLHvuv-SLDDYKGB3L9LphOLWXbExKmexjfRpGIVK0XG0UcsHGpx50GQq69_e0ZHS62IfgjEUISanpdrPi-qo4o3Ggg-8S00FmBFRZDbXpbHevinwEQIKF0KXHDosdtDEzZmg3h2UrgJ4oak2_vuilyWePmMd7g-TqkLkP0rSfXC6EplJezg==&uniplatform=NZKPT&language=CHS
3. Albers, G. J., Amouret, A., Ciupka, K., Cobos, E. M., Feldmann, C., & Reichardt, H. M. (2023). Glucocorticoid nanoparticles show full therapeutic efficacy in a mouse model of acute lung injury and concomitantly reduce adverse effects. *International Journal of Molecular Sciences*, 24(23). <https://doi.org/10.3390/ijms242316843>
4. Almeida, L., Bacon, A., Ghorasaini, M., Ham, A. J. v. d., Toes, R. E. M., Giera, M., & Everts, B. (2025). IgA2 ACPA drives a hyper-inflammatory phenotype in macrophages via ATP synthase and COX2. *European Journal of Immunology*, 55(4), e202451586. <https://doi.org/10.1002/eji.202451586>
5. Chen, Z. H., Li, J. J., Liu, C. J., Chen, D. J., Yang, Y. D., Xie, T. Y., Liang, W. T., Tu, X. P., Shi, W., Yang, X., Chen, Z. F., & Zhang, F. X. (2025). Lan-Qin oral liquid alleviates influenza A virus-induced acute lung

- injury by regulating monocyte-derived macrophages and TLR4/PI3K/AKT/NF- κ B signal pathways. *Journal of Ethnopharmacology*, 347, 119787. <https://doi.org/10.1016/j.Jep.2025.119787>
6. Choudhary, R., Kumar, P., Shukla, S. K., Bhagat, A., Anal, J. M. H., Kour, G., & Ahmed, Z. (2025). Synthesis and potential anti-inflammatory response of indole and amide derivatives of ursolic acid in LPS-induced RAW 264.7 cells and systemic inflammation mice model: Insights into iNOS, COX2 and NF- κ B. *Bioorganic Chemistry*, 155, 108091. <https://doi.org/10.1016/j.Bioorg.2024.108091>
 7. Department of Analytical Chemistry, A. C., Molecular Modelling, V. U. B., Laarbeeklaan 103, Brussels, Belgium., Department of Pharmaceutical Chemistry, D. A., Drug Information, C. f. N., Vrije Universiteit Brussel, Laarbeeklaan 103, Brussels, Belgium., Department of Analytical Chemistry, A. C., Molecular Modelling, V. U. B., Laarbeeklaan 103, Brussels, Belgium., Department of Analytical Chemistry, A. C., Molecular Modelling, V. U. B., Laarbeeklaan 103, Brussels, Belgium., Department of Analytical Chemistry, A. C., Molecular Modelling, V. U. B., Laarbeeklaan 103, Brussels, Belgium., Department of Pharmaceutical Chemistry, D. A., & Drug Information, C. f. N., Vrije Universiteit Brussel, Laarbeeklaan 103, Brussels, Belgium. (2019). Analytical techniques for metabolomic studies: a review. *Bioanalysis*, 11(24), 2297–2318. <https://doi.org/10.4155/bio-2019-0014>
 8. DucDat, L., Sanghee, H., Hyun, M. K., & Mina, L. (2023). Anti-inflammatory activity of compounds derived from Vitex rotundifolia. *Metabolites*, 13(2), 249. <https://doi.org/10.3390/metabo13020249>
 9. Feng, J., Shen, C., Tian, S., Chen, X., Zhou, F., Zhou, M., Zhu, B., Li, X., & Ding, Z. (2024). Total flavonoids from the aerial parts of Tetrastigma hemsleyanum prevent LPS-induced ALI by modulating the TLR4/NF- κ B pathway in mice. *Fitoterapia*, 178, 106175. <https://doi.org/10.1016/j.Fitote.2024.106175>
 10. Feng, Z., Zhenye, G., Fushan, H., Wei, F., Binqiang, W., & Zhonglai, Q. (2021). Magnoflorine alleviates “M1” polarized macrophage-induced intervertebral disc degeneration through repressing the HMGB1/Myd88/NF- κ B pathway and NLRP3 inflammasome. *Frontiers in Pharmacology*, 12, 701087. <https://doi.org/10.3389/fphar.2021.701087>
 11. J, B. K., Ravi, T., & F, L. J. (2010). Pharmacotherapy for prevention and treatment of acute respiratory distress syndrome: current and experimental approaches. *Drugs*, 70(10), 1255–1282. <https://doi.org/10.2165/10898570-000000000-00000>
 12. Lee, H., Jung, K.-H., Lee, H., Park, S., Choi, W., & Bae, H. (2015). Casticin, an active compound isolated from Vitex Fructus, ameliorates the cigarette smoke-induced acute lung inflammatory response in a murine model. *International Immunopharmacology*, 28(2), 1097–1101. <https://doi.org/10.1016/j.intimp.2015.07.041>
 13. Li, W., Song, S., Li, W., & Chen, J. (2025). Flavonoid-rich extract from bombyx batryticatus alleviate LPS-induced acute lung injury via the PI3K/MAPK/NF- κ B pathway. *International Journal of Molecular Sciences*, 26(24), 12057. <https://doi.org/10.3390/ijms262412057>
 14. Li, Y., Zhao, Y., Fang, Q., Guo, R., Shang, G., Zhang, C., Yao, T., Shen, J., Shi, Y., Gao, X., & Qin, K. (2025). Qualitative analysis of chemical components in Viticis Fructus and processed Viticis Fructus by

<https://doi.org/10.1016/j.Microc.2025.113304>

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- experiment. *Russian Military Medical Academy Reports*, 41(2), 111–116.
<https://doi.org/10.17816/rmmar104619>
24. Wang, H., Qiao, L., Zhang, X., Liu, J., Cui, W., Mou, L., Yang, G., Xing, L., Zhang, Z., & Wang, H. (2024). Exploring the mechanism of LPS-induced acute lung injury based on the METTL3/NF- κ B/NLRP3 pathway. *Minerva Surgery*. <https://doi.org/10.23736/s2724-5691.24.10578-3>
 25. Xin, W., Zi-Yi, W., Jia-Hui, Z., & Shao, L. (2021). TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. *Chinese Journal of Natural Medicines*, 19(1), 1–11. [https://doi.org/10.1016/s1875-5364\(21\)60001-8](https://doi.org/10.1016/s1875-5364(21)60001-8)
 26. Xingxu, S., Haixin, X., Zhong, L., He, X., Xin, L., Wenqiao, G., Lihong, P., Lizhi, Z., Jingchun, Y., Chenghong, S., & Guimin, Z. (2023). Jingfang Granules alleviates bleomycin-induced acute lung injury through regulating PI3K/Akt/mTOR signaling pathway. *Journal of Ethnopharmacology*, 318(PA), 116946. <https://doi.org/10.1016/j.Jep.2023.116946>
 27. Xun, G., Yue, Z., Tiantian, F., Lei, C., Wenjing, W., & Kunming, Q. (2021). Comprehensive identification, fragmentation pattern, and metabolic pathways of gefitinib metabolites via UHPLC-Q-TOF-MS/MS; study of rat plasma, urine, bile, and faeces. *Xenobiotica*, 51(3), 355–365.
<https://doi.org/10.1080/00498254.2020.1859642>
 28. Yasmeen, B., Anna, K., & Craig, A. T. (2016). Acute lung injury: A clinical and molecular review. *Archives of Pathology and Laboratory Medicine*, 140(4), 345–350. <https://doi.org/10.5858/arpa.2015-0519-RA>
 29. Zhang, Y., Yuan, C., Wu, Q., Wu, Y., Huang, B., Liu, W., Lv, W., Yu, C., & Chen, L. (2026). Identification of the chemical constituents in Vitis Fructus from different regions by UPLC-QTOF-MS combined with multivariate statistical analyses. *Rapid Communications in Mass Spectrometry: RCM*, 40(1), e10144.
<https://doi.org/10.1002/rcm.10144>
 30. Zhang, Z., Li, W., Shang, Y., Ma, J., Zhang, K., Lin, W., Chen, L., Zhu, Y., Ouyang, H., & He, J. (2026). Multichemical analytical strategy for screening key differential compound between raw and processed Vitis Fructus. *ACS Omega*, 11(6), 9340–9353.
<https://doi.org/10.1021/acsomega.5c09168>
 31. Zhang, Z., Zhang, K., Li, W., Lv, Z., Chen, L., Ma, J., Lin, W., Zhu, Y., Ouyang, H., & He, J. (2026). Comparative pharmacokinetics of sixteen components following oral administration of raw and processed Vitis Fructus in rats. *Natural Product Research*, 1–10.
<https://doi.org/10.1080/14786419.2026.2614399>

Figures

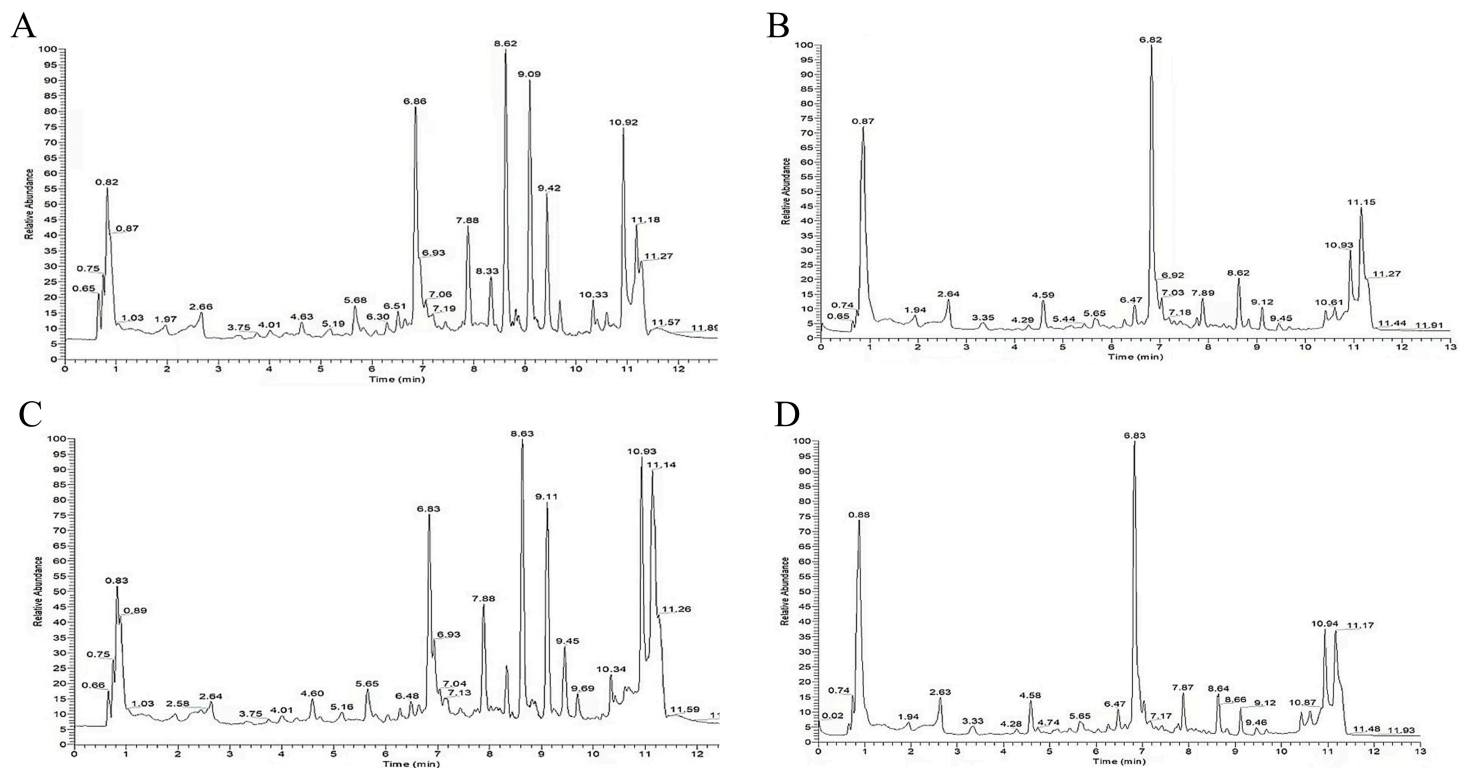


Figure 1

Total ion chromatograms (TICs) for VF (A: positive ion mode; B: negative ion mode) and PVF (A: positive ion mode; B: negative ion mode)

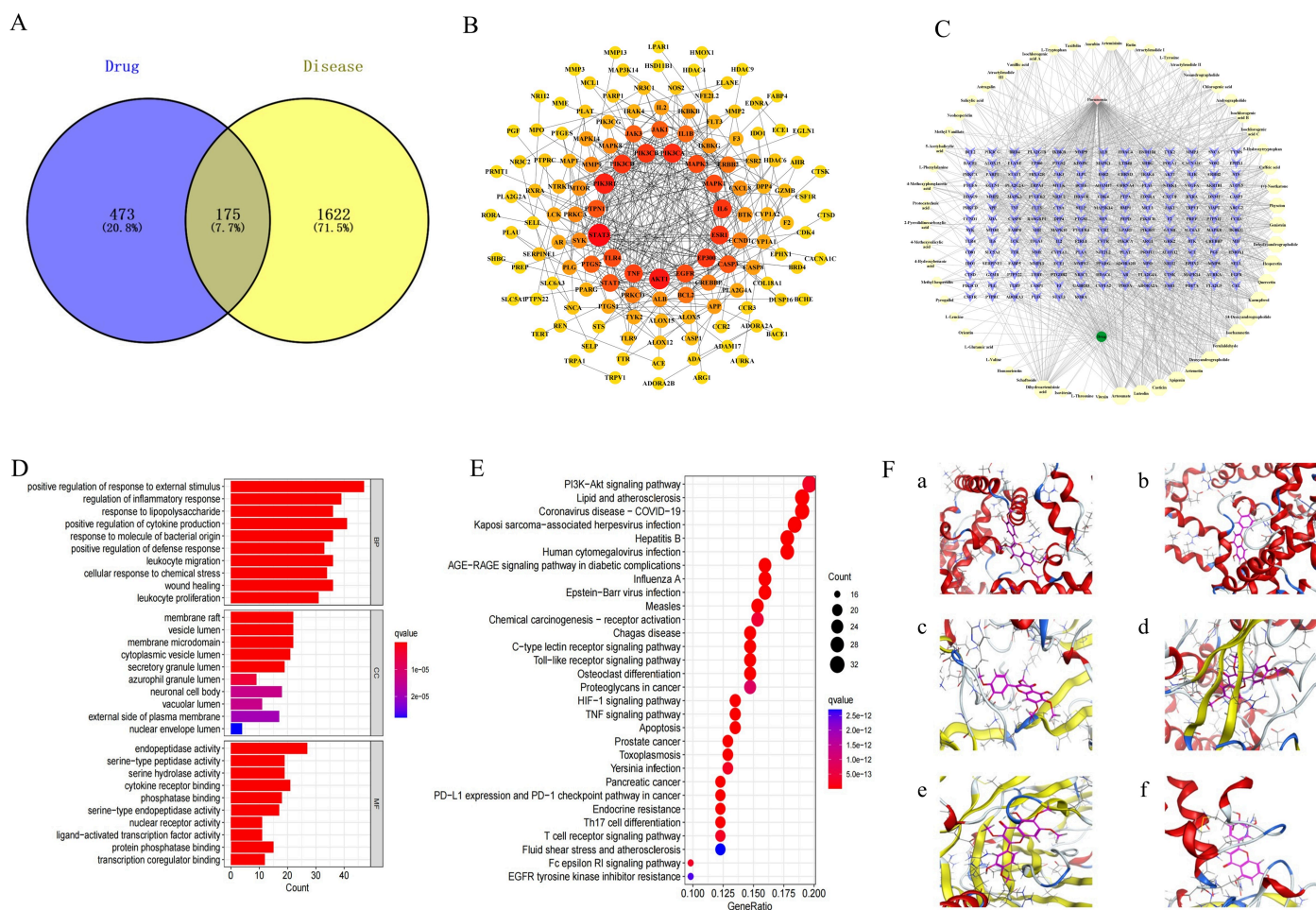


Figure 2

Prediction of the potential mechanism of VF and PVF treatment of ALI. (A) Venn diagram of drug active components and disease targets. (B) PPI network. The color depth represents p-value, and the darker the red, the more significant it is. (C) Network analysis of compound-target interactions, yellow nodes represent traditional Chinese medicine, red nodes represent compounds, blue nodes represent targets, and purple nodes represent signal pathways. (D) GO enrichment analysis of VF. The color depth represents p-value, and the darker the red, the more significant it is. (E) KEGG pathway analysis of potential treatment targets of VF. The color depth represents p-value, and the darker the red, the more significant it is. (F) Molecular docking diagrams. (a) 3D molecular docking of Casticin and STAT3; (b) Luteolin and STAT3; (c) Casticin and AKT1; (d) Luteolin and AKT1; (e) Casticin and IL6; (f) Luteolin and IL6

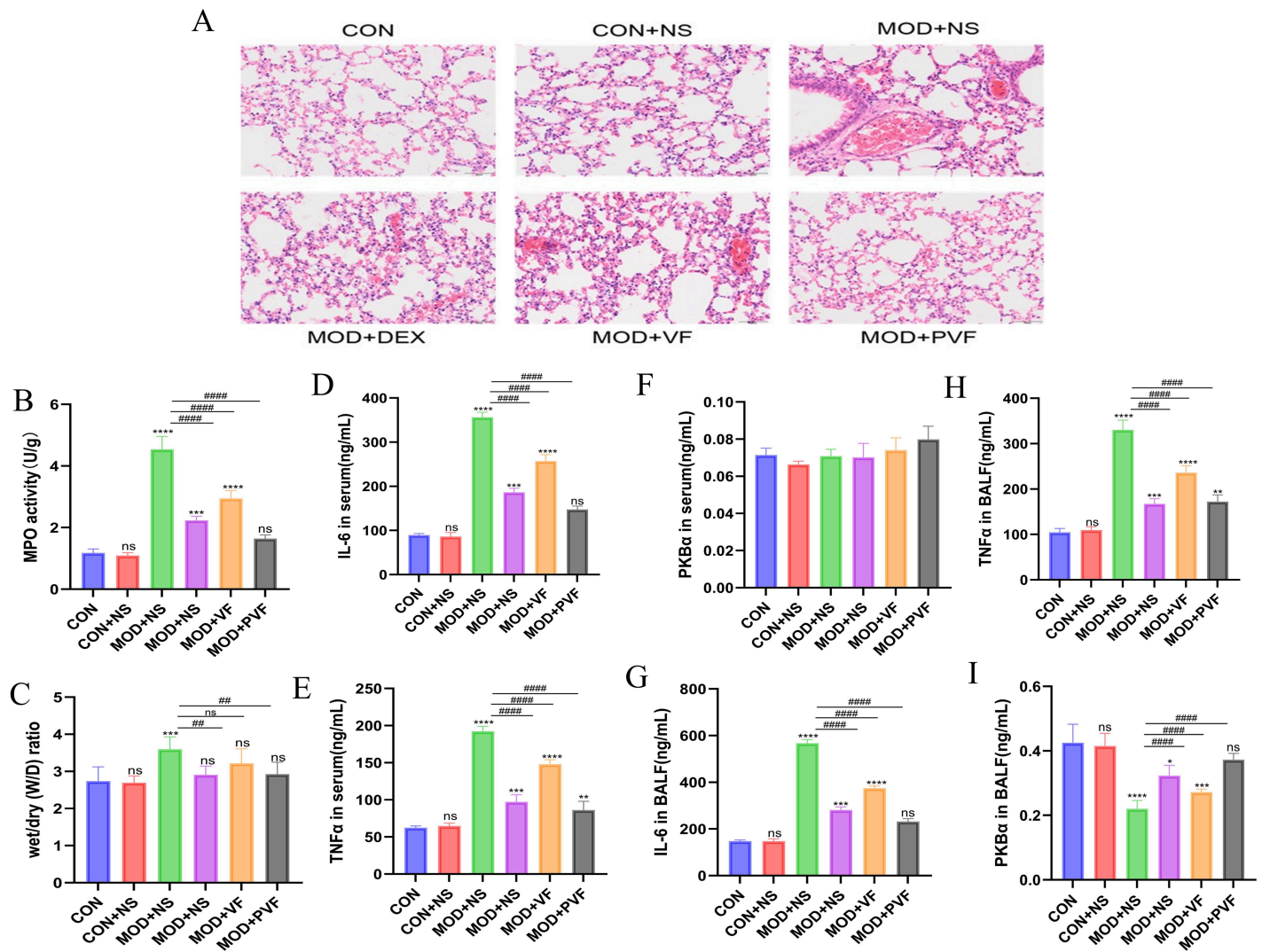


Figure 3

Protective effects of VF and PVF against LPS-induced ALI in vivo. (A) Lung tissue sections stained with H&E for histopathological analysis (magnification: 400×, n = 6). (B) MPO activity in lung tissue. (C) Lung wet/dry ratio; VF and PVF inhibit inflammatory responses in LPS-induced ALI in mice. (D-I) Levels of inflammatory cytokines (IL-6, TNF-α, PKBα) in serum and lung tissues. Data are expressed as mean ± SD. ***p < 0.001, ****p < 0.0001 vs. CON group; ##p < 0.01, ####p < 0.0001 vs. MOD group

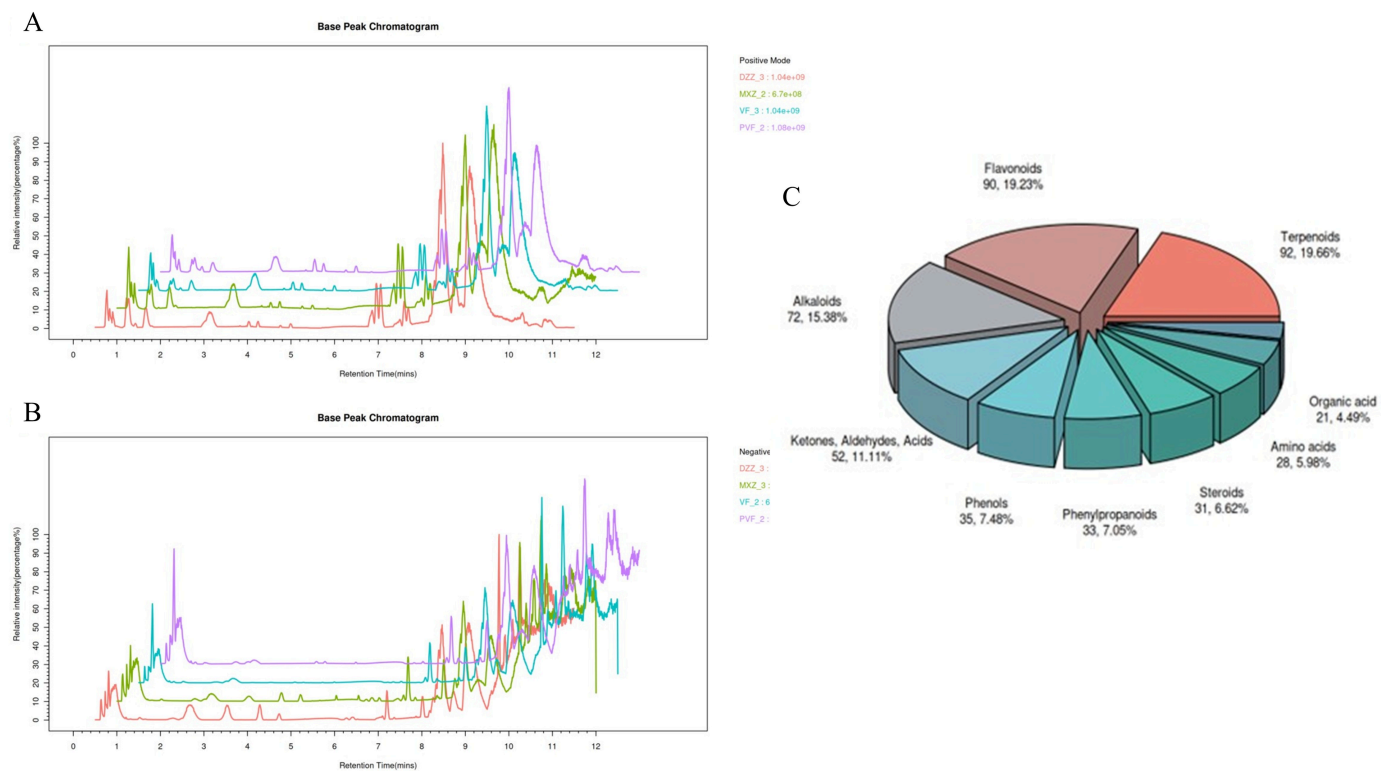


Figure 4

TICs of VF (A) and PVF (B). (C) Classification of metabolites identified in mouse lung tissue

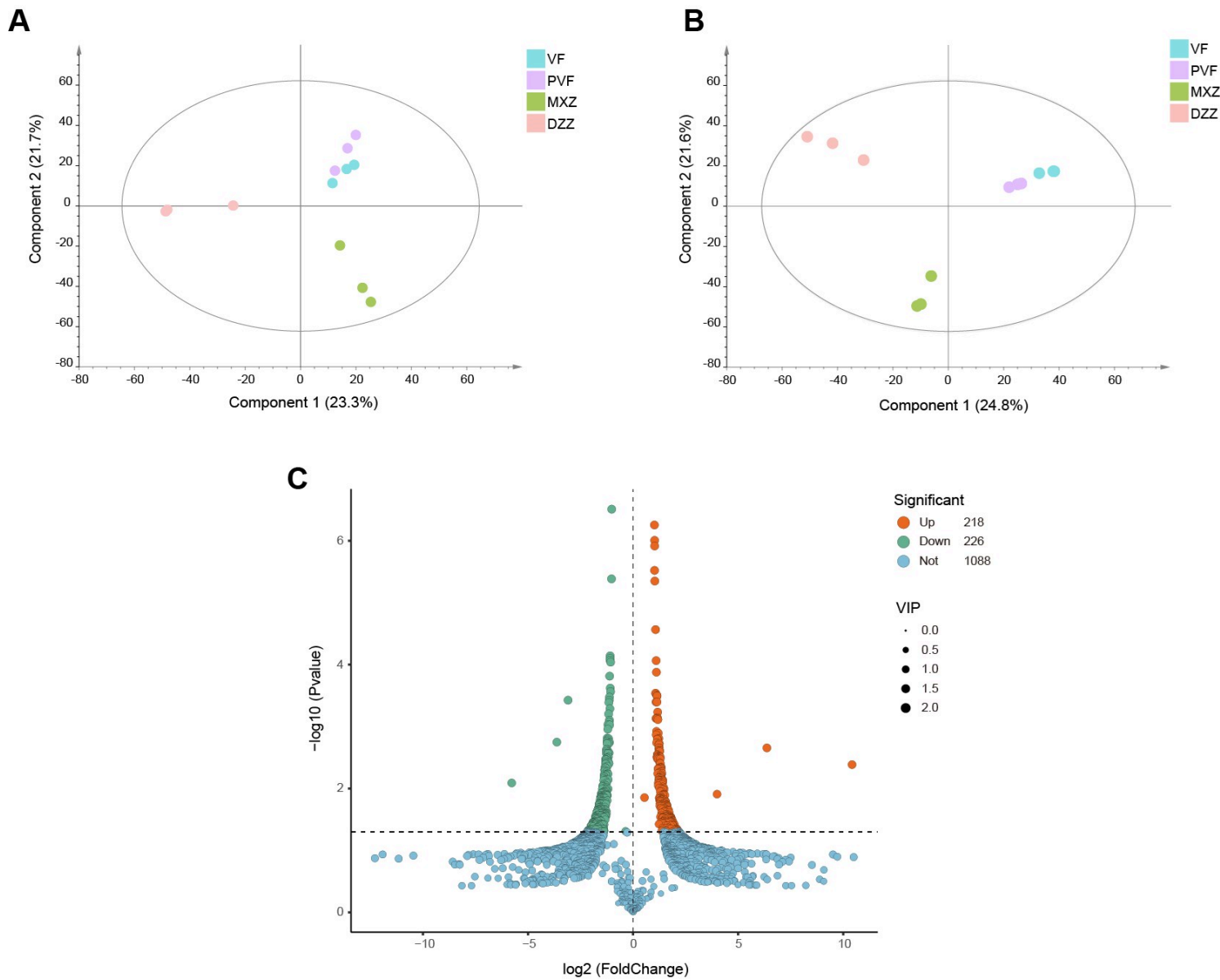


Figure 5

Metabolomic characteristics and differential metabolite analysis of mouse lung tissue (n = 3). (A-B) OPLS-DA score plots showing metabolic profiles of the CON, MOD, VF, and PVF groups. (C) Volcano plot of metabolite changes

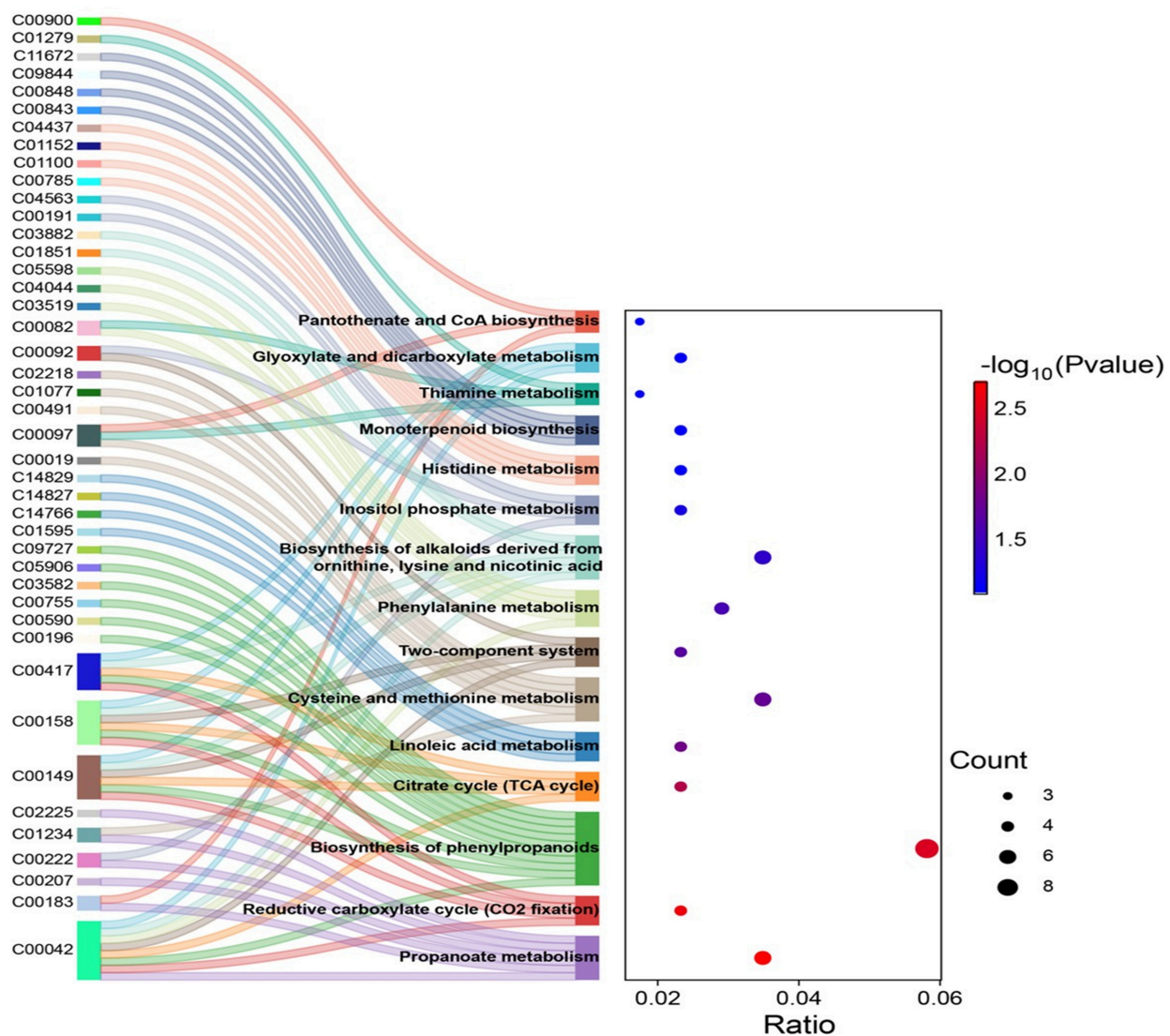


Figure 6

KEGG pathways identified by metabolomics

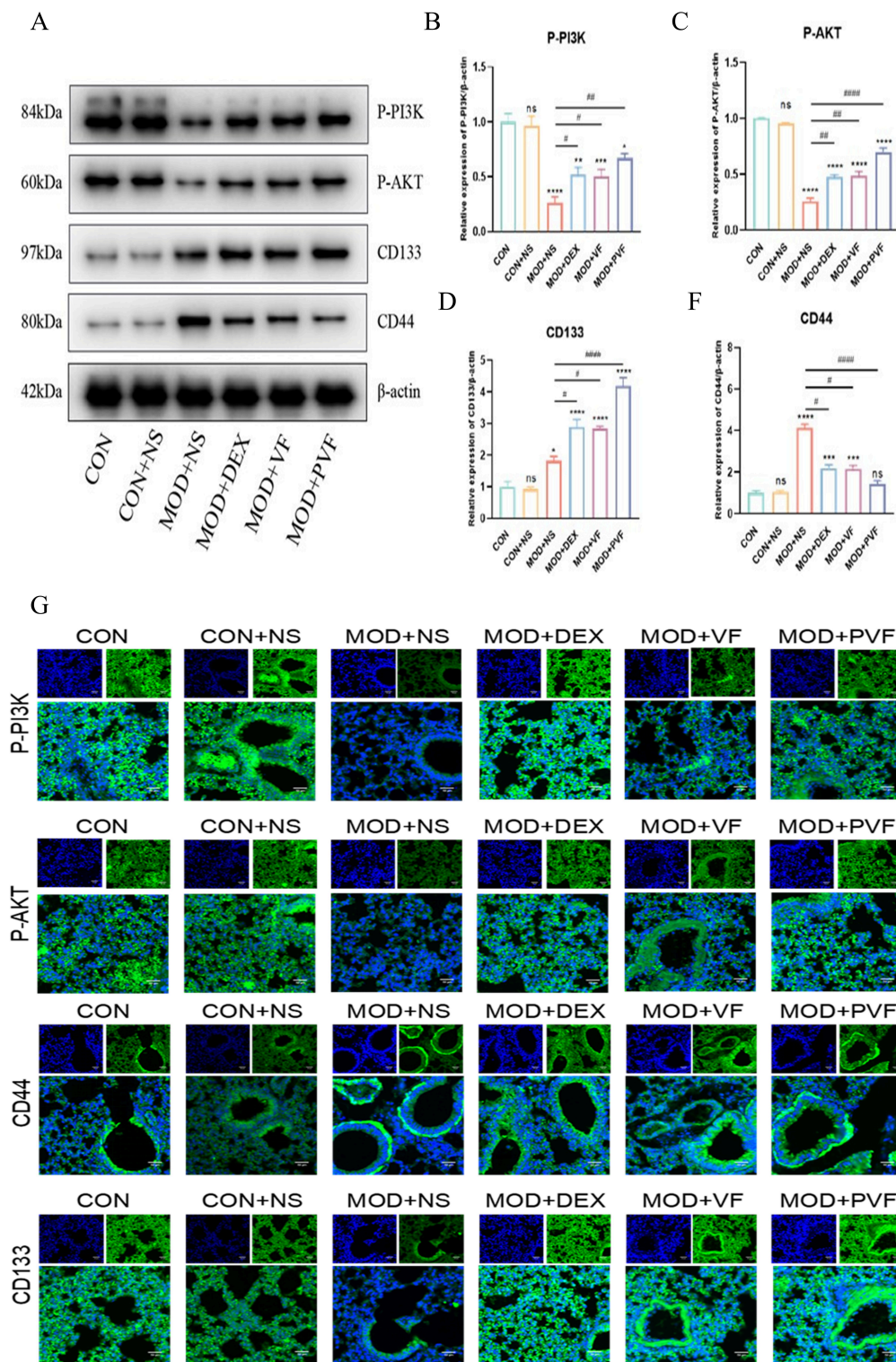


Figure 7

Effects of VF and PVF on the expression of P-PI3K, P-AKT, CD133, and CD44 (A), and the ratios of P-PI3K/ β -actin (B), P-AKT/ β -actin (C), CD133/ β -actin (D), and CD44/ β -actin (E); Immunofluorescence analysis of P-PI3K, P-AKT, CD44, CD133 (G) in hypothalamus tissue. Data are expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. CON group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ vs. MOD group

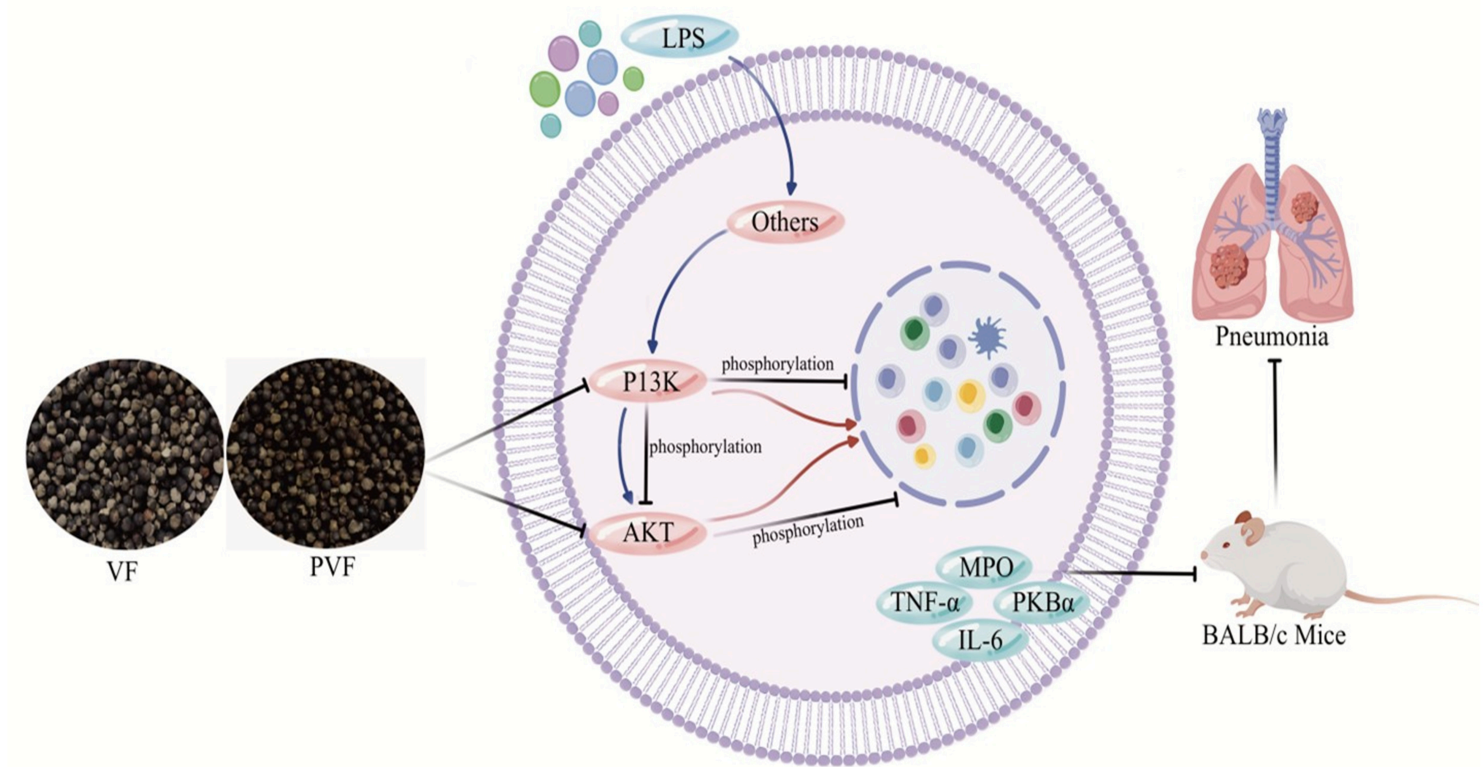


Figure 8

Main active mechanisms of raw VF and PVF against LPS-induced pneumonia in mice

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