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Exploring the Mechanism of "Radix Bupleuri-Paeoniae Radix Alba" in the Treatment of Post-Stroke Depression Based on Network Pharmacology and Molecular Docking

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Keywords

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Abstract

Purpose: To explore the mechanism of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of post-stroke depression through network pharmacology and molecular docking. **Methods:** The active ingredients and targets of "Radix Bupleuri-Paeoniae Radix Alba" were predicted by Traditional Chinese Medicine Systems Pharmacology (TCMSP) database. The related targets of post-stroke depression were searched in GeneCards, NCBI and DisGeNET databases. Cystoscape software was used to construct the "Chinese Medicine-Active Ingredient-Disease-Intersection Target" network, followed by Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The active ingredients corresponding to the core targets were verified by molecular docking techniques. **Results:** A total of 145 potential core targets of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of post-stroke depression were identified. The core ingredients such as quercetin, kaempferol and β-sitosterol, and the key protein targets such as RAC-alpha serine/threonine-protein kinase 1 (AKT1), Interleukin-6 (IL-6) and Interleukin-1β (IL-1β) were screened out. KEGG pathway enrichment analysis mainly involved "lipids and atherosclerosis", "chemical carcinogenesis-receptor activation", and "hepatitis B". Molecular docking results showed that quercetin and kaempferol, the main active ingredients of "Radix Bupleuri-Paeoniae Radix Alba", had good affinity with the key potential targets AKT1, IL-6 and IL-1β. **Conclusion:** The pair of "Radix Bupleuri-Paeoniae Radix Alba" may act on the core targets such as AKT1, IL-6 and IL-1β through the main active ingredients including quercetin and kaempferol, and may effectively treat post-stroke depression by regulating multiple signaling pathways such as "lipids and atherosclerosis" and "hepatitis B".

1 Introduction

Stroke is one of the top three diseases in terms of mortality globally and has nearly 6 million deaths annually, a number that is expected to reach 12 million by 2030 [1]. Post-stroke depression (PSD) is defined as depression state after the onset of stroke [1], which is a severe complication of stroke and has gradually become one of the commonest neuropsychological complications of stroke, resulting in declined life quality and rehabilitation ability. PSD can occur in the acute, recovery, and sequelae stages of stroke, and during difference follow-up periods, the incidence of PSD ranges from 20% to 43% [2]. The primary clinical symptoms of PSD include low mood, loss of interest, emptiness, anxiety, irritability, pessimism, sleep disorders, general fatigue, etc. [3,4]. There are multiple causes for the occurrence of PSD, such as female, age (<70 years), family history, nervousness, severity of stroke and disability, anxiety, aphasia, cognitive ability, and chronic stress (including financial and health-related stress) [5-7]. Also, PSD is an important factor for the recurrence of stroke, which not only seriously affects the quality of life of patients, but also imposes a serious burden on stroke families and society.

Currently, the underlying physiological and pathological mechanism of PSD remains unclear [8], but the possible mechanisms consist of changes in the upstream monoamine pathway, excessive pro-inflammatory cytokines, dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, and alterations in neuroplasticity according to heaps of research [1]. The treatment of PSD mainly comprises selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and other antidepressants [1]. However, these antidepressants have multiple side effects, such as dizziness, drowsiness, and anticholinergic side effects [9]. Moreover, there exists some controversy over the effectiveness of antidepressants in improving the quality of life, function, and cognitive outcomes of patients with neurological disorders [10]. To minimize side effects and maximize therapeutic efficacy, alternative treatment methods are sorely needed [11].

From the perspective of traditional Chinese medicine (TCM), PSD belongs to concurrent meridian pattern of “wind stroke” and “qi stagnation”, which, based on wind stroke, is a depressive manifestation resulting from blood stasis and qi stagnation, as well as obstructed qi in liver due to the stagnation of wind, phlegm, fire and blood stasis [12]. Hence, despite the disease location in the brain, PSD has the closest relationship with the liver [13]. “Radix Bupleuri-Paeoniae Radix Alba” is the most fundamental medicine pair for “soothing the liver, resolving depression, and regulating emotions”, which forms the basis of formulas such as Xiao Yao San, Si Ni San, and Xiao Chai Hu Tang in the Bupleurum category. A previous study explored the medication rules of TCM for PSD on the Chinese medicine inheritance auxiliary platform and found that “Radix Bupleuri-Paeoniae Radix Alba” is one of the top five most frequently used drug combinations targeting PSD [14]. Radix Bupleuri is a commonly used drug for soothing the liver, and the Transforming the Significance of Medicinal Substances recorded that “Radix Bupleuri is light and clear in nature governing ascending and dispersing, and is slightly bitter in taste mainly for soothing the liver” [15]. Paeoniae Radix Alba is bitter and sour in taste, and slightly cold in nature, which can nourish blood, consolidate Yin, soften the liver, and relieve pain. The combination of these two drugs has the function of dispersing and consolidating, and nourishing both qi and blood, exerting synergetic effects [16]. Modern pharmacological studies have shown that Radix Bupleuri has pharmacological effects such as antipyretic, analgesic, anti-inflammatory, and antidepressant properties [17], while Paeoniae Radix

Alba possesses analgesic, anti-inflammatory, antidepressant, anti-cardiovascular/cerebrovascular diseases, and anti-neurodegenerative diseases effects [18]. Reportedly, the Radix Bupleuri-Paeoniae Radix Alba combination is significantly effective in treating PSD [19], but the relevant specific material basis and mechanism are not yet clarified. This study utilizes network pharmacology analysis to screen the core active ingredients, key targets, and main signaling pathways of "Radix Bupleuri-Paeoniae Radix Alba" in treating PSD, and leverages molecular docking methods to verify the binding of active ingredients and key targets, to explore the main material basis and mechanism of "Radix Bupleuri-Paeoniae Radix Alba" in treating PSD, providing a specific basis for future in-depth research.

2 Materials and methods

2.1 Screening of active ingredients in Radix Bupleuri-Paeoniae Radix Alba and targets for PSD

Screening of active ingredients and targets of Radix Bupleuri-Paeoniae Radix Alba: All effective active ingredients of Radix Bupleuri-Paeoniae Radix Alba were retrieved on Traditional Chinese Medicine Systems Pharmacology (TCMSP) (https://tcmspw.com/tcmsp.php), with the settings of oral bioavailability (OB) value ⩾ 30% and drug-likeness (DL) ⩾ 0.18. The target prediction of the included compound ingredients was conducted using TCMSP database, and all targets were corrected and converted to symbol name using the Uniprot database (https://www.uniprot.org/) with non-human targets removed.

Screening of targets of PSD: With "Post stroke depression" as key words, relevant targets of PSD were searched in GeneCards (https://www.genecards.org/), NCBI (https://www.ncbi.nlm.nih.gov/) and DisGeNET (https://www.disgenet.org/) databases. The results from the three databases were integrated and the duplicated gene targets were deleted, yielding PSD-related targets.

2.2 Construction of intersection of drug and disease-related targets

To further anatomize the relation between "Radix Bupleuri-Paeoniae Radix Alba" and PSD, the selected drug targets and disease targets were input in Venn 2.1 for drawing a Venn diagram of the relationship between "Radix Bupleuri-Paeoniae Radix Alba" and PSD-related targets. The common targets were identified and acted as predictive targets for the effect of "Radix Bupleuri-Paeoniae Radix Alba" on PSD.

2.3 Construction of "TCM-active ingredients-disease-intersection targets" network and screening of core active ingredients

With the obtained intersection targets of drug and disease in Section 2.2 and through collating TCM, active ingredients and disease information, Cytoscape 3.8.0 was exploited to establish "TCM-active ingredients-disease-intersection targets" network [20]. Topology analysis was performed using Network Analyzer tool and the core ingredients of "Radix Bupleuri-Paeoniae Radix Alba" for treating PSD were selected based on topology parameters.

2.4 Construction of protein-protein interaction (PPI) network diagram and screening of core proteins

The common targets of drug and diseases were input into the String database (https://string-db.org/cgi/input.pl) for constructing a PPI network [21]. With "Homo sapiens" as biological species. The hide disconnected nodes were removed from the network and the minimum required interaction score was set as high confidence of 0.900 to get the data of PPI. Then, the data of PPI was imported into Cytoscape to draw the PPI network, and the top 10 core proteins were obtained by R 4.1. Core targets were filtered through three key topological parameters, including degree centrality (DC), closeness centrality (CC) and

betweenness centrality (BC). The values of these three parameters indicate the importance and influence of related nodes in the network. The cutoff value was set to the median. PPI network were filtered by molecular complex detection algorithm (MCODE) plugin in Cytoscape. Herein, degree was set to 2, k-core was set to 2, max depth was set to 0.2, and maximum depth was set to 100.

2.5 GO function and KEGG pathway enrichment analysis

The common targets of drug and diseases underwent KEGG pathway enrichment analysis and GO function enrichment analysis from three levels of biological process (BP), molecular function (MF) and cell component (CC).

Utilizing the String database, the items with a corrected p value of less than 0.05 were filtered. With R 4.0.3 software and the packages clusterProfiler, enrichplot, and ggplot2 installed, the bar and bubble charts were plotted. To more vividly illustrate the multi-ingredient and multi-target characteristics of active ingredients in TCM during the treatment process, the ingredient-disease-pathway-target network files were imported into Cytoscape 3.8.0 for the generation of pathway network diagrams.

2.6 Molecular docking of key targets and core active ingredients

3D structure of core proteins in pathway was retrieved in RCSB Protein Data Bank database (https://www.rcsb.org/). Through the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/), the structural file of the main active ingredients in SDF format was downloaded and imported into Open Babel 2.4.1 software to convert 2D small molecule compounds into 3D structures in mol2 format. Using AutoDock Tools 1.5.6 software, the proteins and small molecule compounds underwent dehydration and hydrogenation, the charge was calculated, and non-polar hydrogen was combined. The results were visualized in PyMOL Molecular Graphics System software.

3 Results

3.1 Screening of active ingredients and targets in Radix Bupleuri-Paeoniae Radix Alba

Due to multi-ingredient and multi-target characteristics of TCM, it is necessary to filter active compounds. After summarizing and deleting duplicates through retrieval in TCMSP database and absorption, distribution, metabolism and excretion (ADME) parameter screening, we obtained 17 compound ingredients and 179 targets of Radix Bupleuri, as well as 13 compound ingredients and 86 targets of Paeoniae Radix Alba. Among them, kaempferol is an active ingredient both existing in Radix Bupleuri and Paeoniae Radix Alba and there were a total of 29 active ingredients of “Radix Bupleuri-Paeoniae Radix Alba” (Table 1).

3.2 Information about PSD-related targets and obtainment of “TCM-disease” intersection targets

The retrieval on GeneCards, NCBI and DisGeNET databases revealed 2535, 151 and 39 PSD-related targets, respectively. After we integrated, de-duplicated and standardized the genes from these three databases, a total of 2544 PSD-related targets were obtained. The “Radix Bupleuri-Paeoniae Radix Alba” targets and disease targets were imported into Venn 2.1, resulting in 145 common targets, which were potential targets in treatment of PSD using “Radix Bupleuri-Paeoniae Radix Alba” medicine pair (Figure 1).

Table 1 Active ingredients of Radix Bupleuri and Paeoniae Radix Alba.

Traditional Chinese medicine	No.	Name	OB/%	DL
Radix Bupleuri	MOL001645	Linoleyl acetate	42.10	0.20
Radix Bupleuri	MOL002776	Baicalin	40.12	0.75
Radix Bupleuri	MOL000449	Stigmasterol	43.83	0.76
Radix Bupleuri	MOL000354	isorhamnetin	49.60	0.31
Radix Bupleuri	MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	31.97	0.59
Radix Bupleuri	MOL004609	Areapillin	48.96	0.41
Radix Bupleuri	MOL013187	Cubebin	57.13	0.64
Radix Bupleuri	MOL004624	Longikaurin A	47.72	0.53
Radix Bupleuri	MOL004628	Octalupine	47.82	0.28
Radix Bupleuri	MOL004644	Sainfuran	79.91	0.23
Radix Bupleuri	MOL004648	Troxerutin	31.60	0.28
Radix Bupleuri	MOL004653	(+)-Anomalin	46.06	0.66
Radix Bupleuri	MOL004702	saikosaponin c_qt	30.50	0.63
Radix Bupleuri	MOL004718	α-spinasterol	42.98	0.76
Radix Bupleuri	MOL000490	petunidin	30.05	0.31
Radix Bupleuri	MOL000098	quercetin	46.43	0.28
Radix Bupleuri and Paeoniae Radix Alba	MOL000422	kaempferol	41.88	0.24
Paeoniae Radix Alba	MOL000492	(+)-catechin	54.83	0.24
Paeoniae Radix Alba	MOL001928	albiflorin_qt	66.64	0.33
Paeoniae Radix Alba	MOL001918	paeoniflorgenone	87.59	0.37
Paeoniae Radix Alba	MOL001910	11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide	64.77	0.38
Paeoniae Radix Alba	MOL001925	paeoniflorin_qt	68.18	0.4
Paeoniae Radix Alba	MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53
Paeoniae Radix Alba	MOL001930	benzoyl paeoniflorin	31.27	0.75
Paeoniae Radix Alba	MOL000359	sitosterol	36.91	0.75
Paeoniae Radix Alba	MOL000358	beta-sitosterol	36.91	0.75
Paeoniae Radix Alba	MOL000211	Mairin	55.38	0.78
Paeoniae Radix Alba	MOL001924	paeoniflorin	53.87	0.79
Paeoniae Radix Alba	MOL001921	Lactiflorin	49.12	0.80

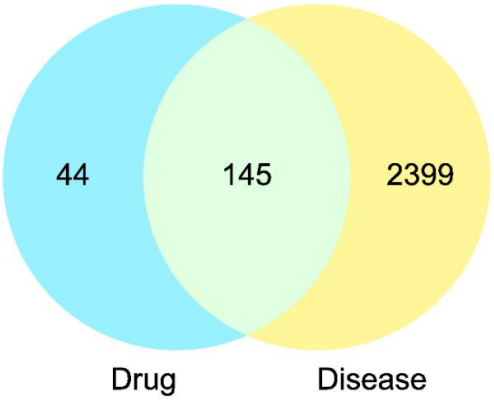


Figure 1 The Venn diagram of "Radix Bupleuri-Paeoniae Radix Alba" medicine pair and PSD targets.

3.3 Construction of "TCM-active ingredients-disease-intersection targets" network and screening of core active ingredients

To unveil the intersection between targets of "Radix Bupleuri-Paeoniae Radix Alba" active ingredients and disease targets, information regarding TCM (Radix Bupleuri-Paeoniae Radix Alba), active ingredients, intersection targets and disease was imported into Cytoscape 3.8.0, constructing "TCM-active ingredients-disease-intersection targets" network (Figure 2). In the figure, the blue purple represents the active ingredients, the green represents the target of the drug acting on the disease, the yellow represents the "Radix Bupleuri-Paeoniae Radix Alba" medicine pair, and the red rectangle represents the disease. The ingredients were sorted by degree, with higher degree values indicating greater importance of the ingredients (Table 2). From Table 2, it can be noticed that the top three targets with high correlation were quercetin, kaempferol and β-sitosterol, among which kaempferol was a common component of Radix Bupleuri and Paeoniae Radix Alba. In Radix Bupleuri, quercetin had the strongest correlation with the target, while in Paeoniae Radix Alba, β-sitosterol had the strongest association with target. Overall, quercetin and kaempferol had the most interactions with their targets and may have relatively greater value for future research.

3.4 Construction of PPI network and screening of core proteins

The potential targets of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD were imported into String database for PPI network construction, with "Homo sapiens" as biological species (Figure 3). The PPI network had 145 nodes, 2378 edges, and mean degree of 33. The PPI network was imported into Cytoscape 3.8.0 to perform topology analysis using the Network Analyzer tool, and the top 20 target genes with the most linked nodes were sorted out to create a bar diagram of the core genes in the PPI network (Figure 4). The core protein target genes included AKT1, IL-6, IL-1β, etc., which provided certain reference value for studying the mechanism of "Radix Bupleuri-Paeoniae Radix Alba" in treating PSD.

3.5 GO function and KEGG pathway enrichment analysis

To clarify the mechanism of active ingredients of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD, we obtained GO function bar diagram (Figure 5) and KEGG pathway enrichment bubble diagram (Figure 6) based on R language. The results revealed that the main enrichment items of potential targets comprised "response to lipopolysaccharide", "response to molecule of bacterial origin", "cellular response to chemical stress", "response to drug" and "response to oxidative stress" in biological processes; "membrane

raft", "membrane microdomain", and "membrane regions" in the cellular component group; and "DNA-binding-transcription factor binding", "RNA polymerase II specific DNA-binding-transcription factor binding", "ubiquitin-like protein ligase binding", etc., in molecular functions.

The results indicated that KEGG pathways with high enrichment included "lipids and atherosclerosis", "chemical carcinogenesis-receptor activation", "Hepatitis B", "AGE-RAGE signaling pathway in diabetes complications", "Kaposi sarcoma associated herpesvirus infection". Besides, "Radix Bupleuri-Paeoniae Radix Alba" regulated PSD via multiple biological processes and signaling pathways. We noticed that "lipids and atherosclerosis" ranked first in KEGG pathway enrichment, and reportedly, atherosclerosis is a risk factor for PSD [22].

The "active ingredients-disease-pathway-targets" network of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD was imported into Cytoscape3.8.0 to establish "TCM-active ingredients-core targets-biological function-signaling pathway" network (Figure 7). As delineated in Figure 7, the blue, pink, green, yellow and purple signify compounds, targets of TCM acting on disease, the most prominent top 20 pathways, diseases, and TCM. The network contributed to visualization of main material basis and mechanism of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD.

Table 2 The core active ingredients of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD.

MOL ID	Name	Degree
MOL000098	quercetin	111
MOL000422	kaempferol	44
MOL000358	beta-sitosterol	31
MOL000354	isorhamnetin	28
MOL000449	Stigmasterol	23
MOL004609	Areapillin	13
MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	9
MOL000490	petunidin	8
MOL000492	(+)-catechin	8
MOL004653	(+)-Anomalin	6
MOL013187	Cubebin	6
MOL001645	Linoleyl acetate	4
MOL001924	paeoniflorin	4
MOL000359	sitosterol	3
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	3
MOL002776	Baicalin	3
MOL004718	α-spinasterol	3
MOL000211	Mairin	2
MOL001918	paeoniflorgenone	2
MOL004624	Longikaurin A	2

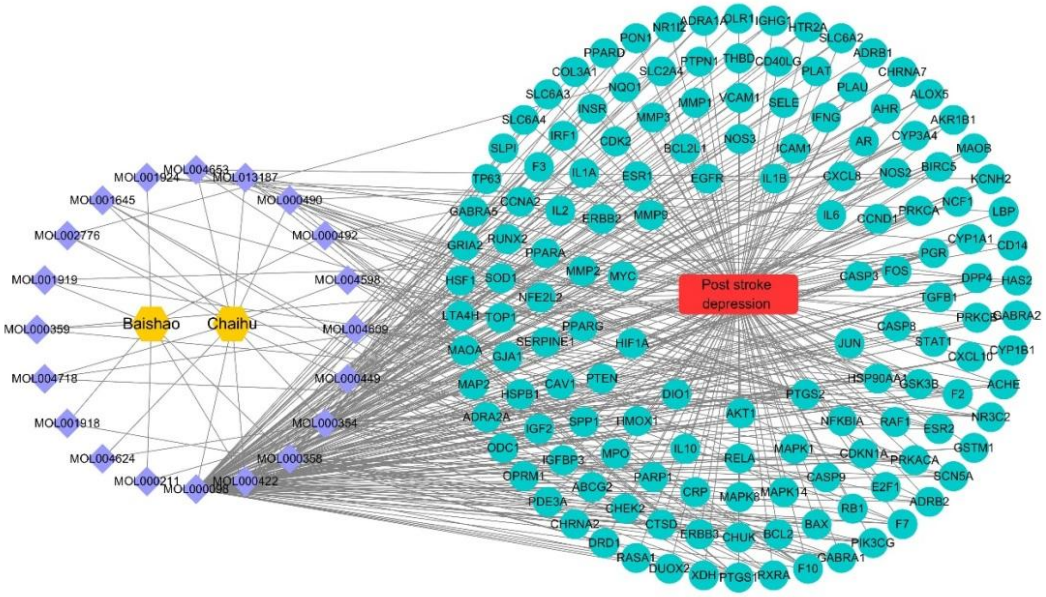


Figure 2 The "TCM-active ingredients-disease-intersection targets" network of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD.

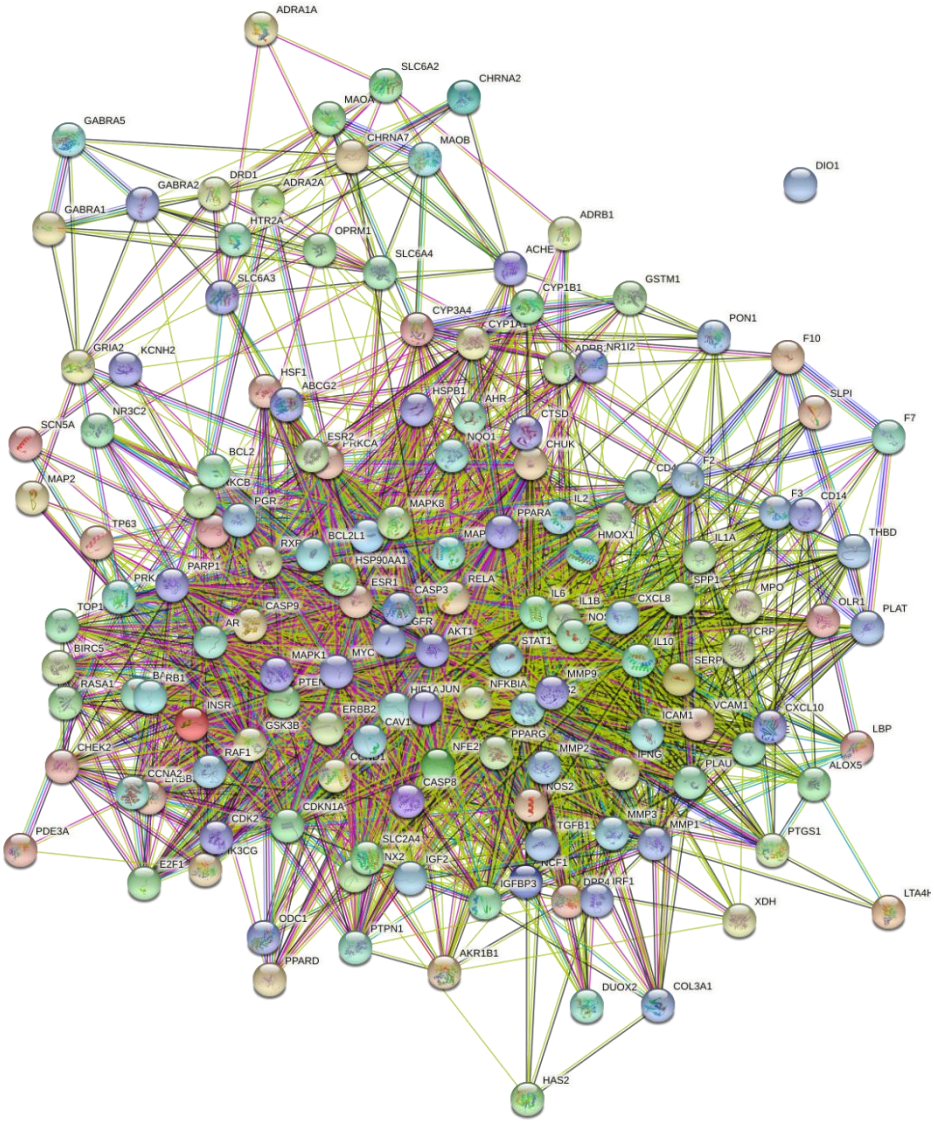


Figure 3 PPI network diagram.

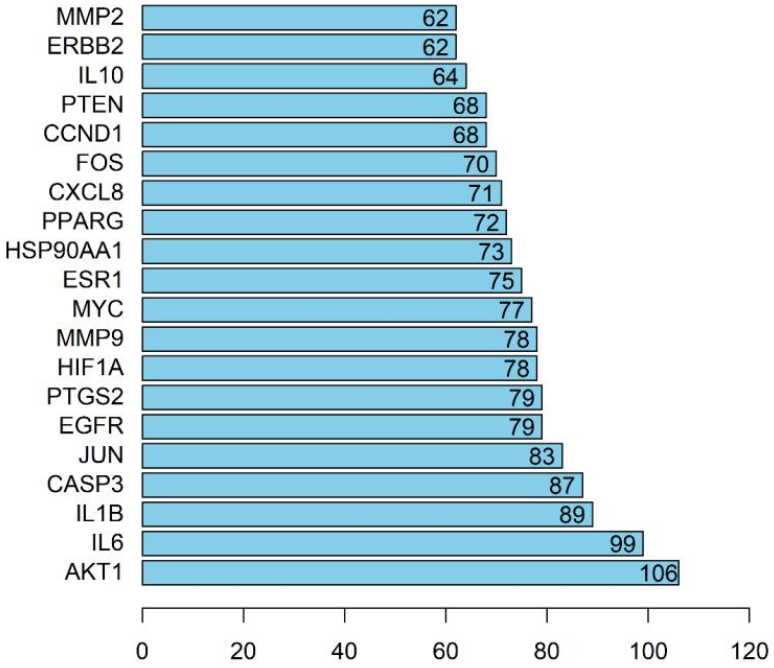


Figure 4 PPI network of proteins of core genes.

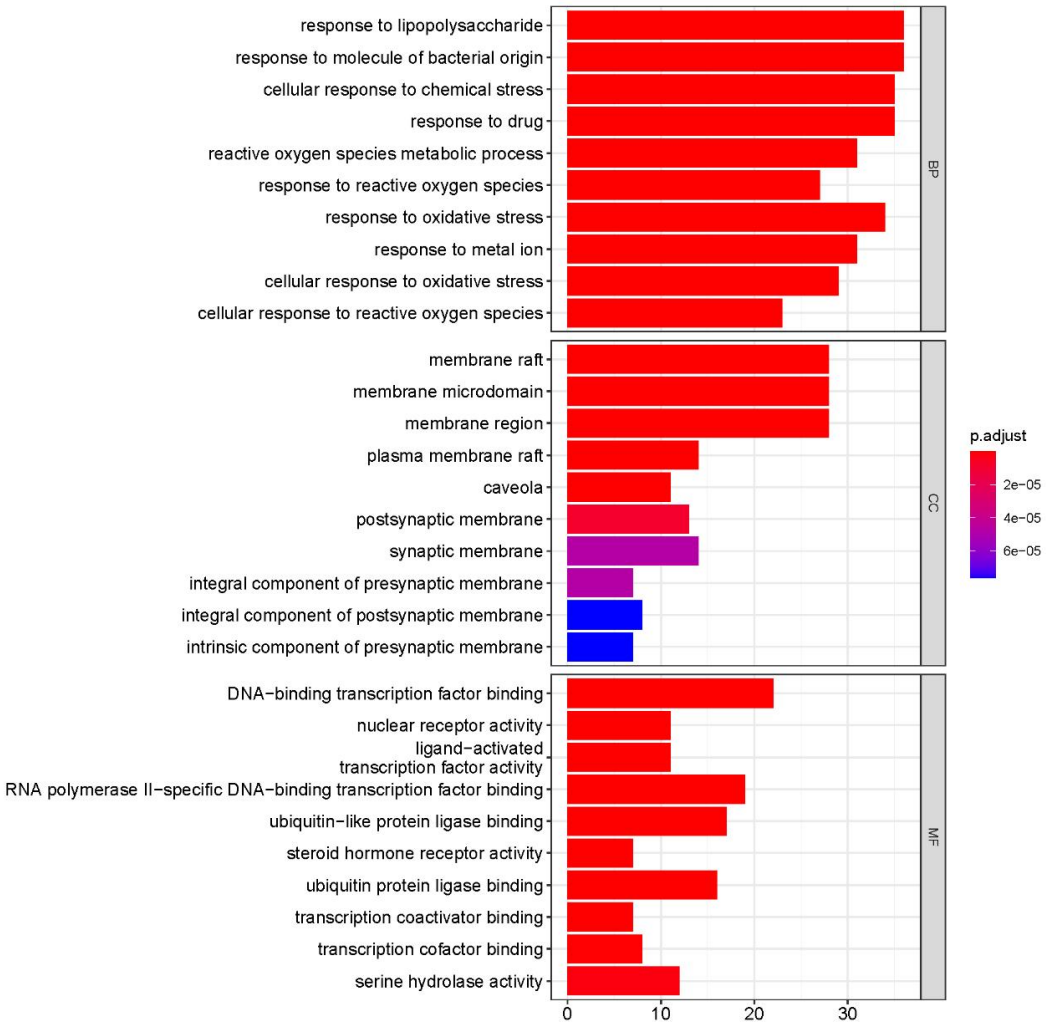


Figure 5 The bar diagram of GO function analysis.

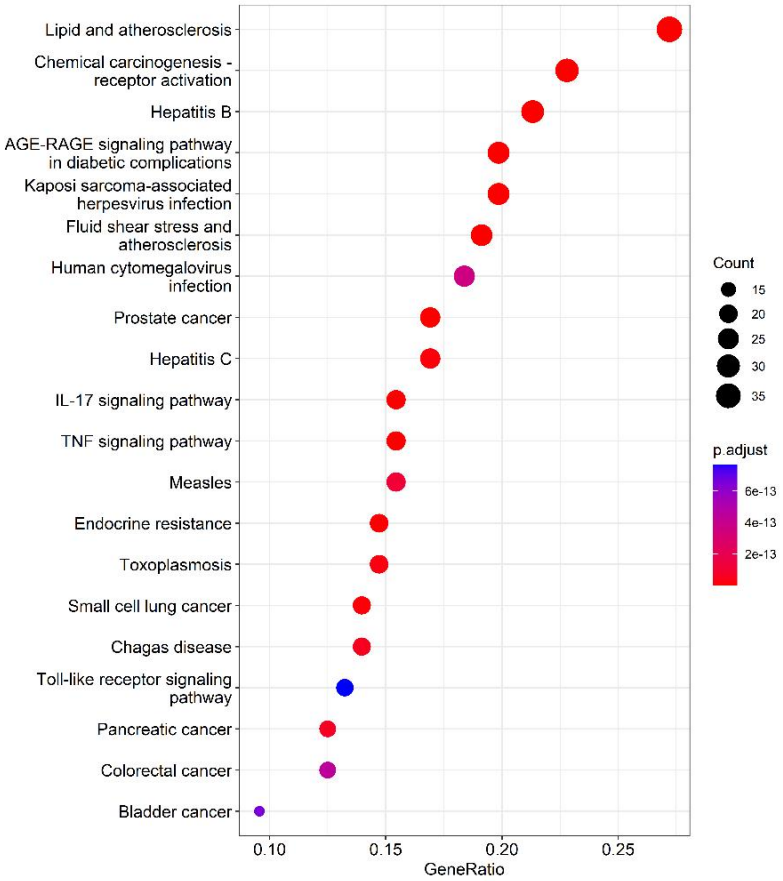


Figure 6 The bubble diagram of KEGG pathway.

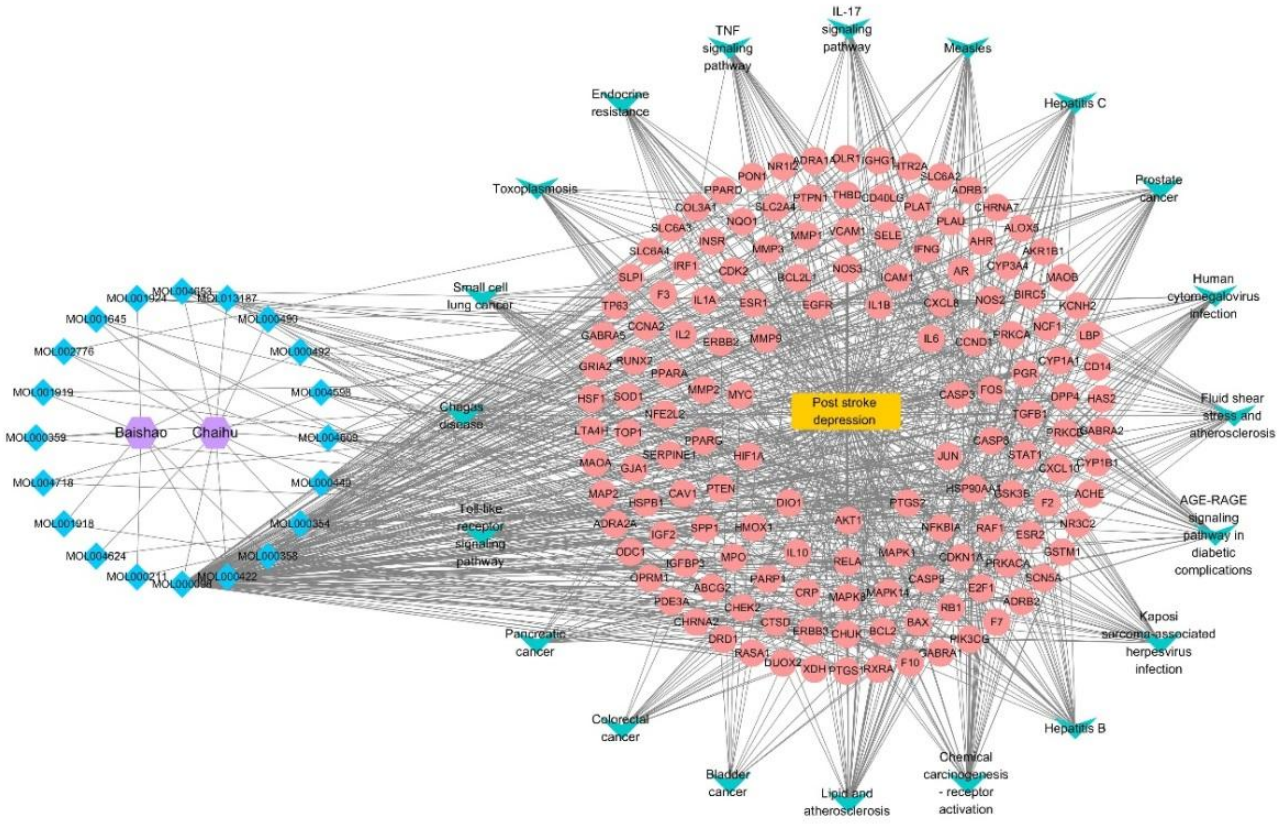


Figure 7 The network of "TCM-active ingredients-core targets-biological function-signaling pathway" of "Radix Bupleuri-Paeoniae Radix Alba" in the treatment of PSD.

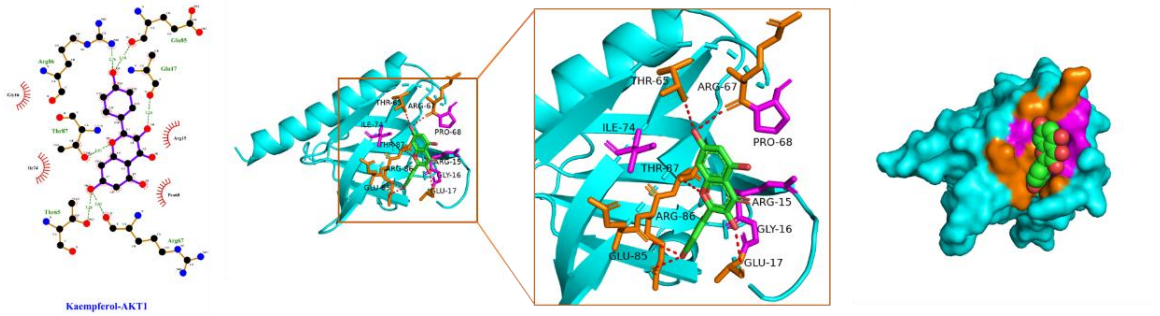
3.6 Molecular docking

Using Sybyl-X 2.0 software, the top 3 core targets and top 2 core ingredients with the highest degree values were selected for molecular docking (Table 3), with the lower binding energy score implying the more stable binding. We confirmed that the binding energy score of the selected core ingredients and the target docking was between -5.0 and -7.0, indicating that the small molecules of the compound had good binding ability to the target. Among them, kaempferol had a good affinity for AKT1, while quercetin had a good affinity for IL-1β, AKT1, and IL-6 proteins. These findings hinted that quercetin and kaempferol played critical roles in the efficacy of "Radix Bupleuri-Paeoniae Radix Alba", which provided pivotal reference for the mechanism of the medicine pair in PSD treatment.

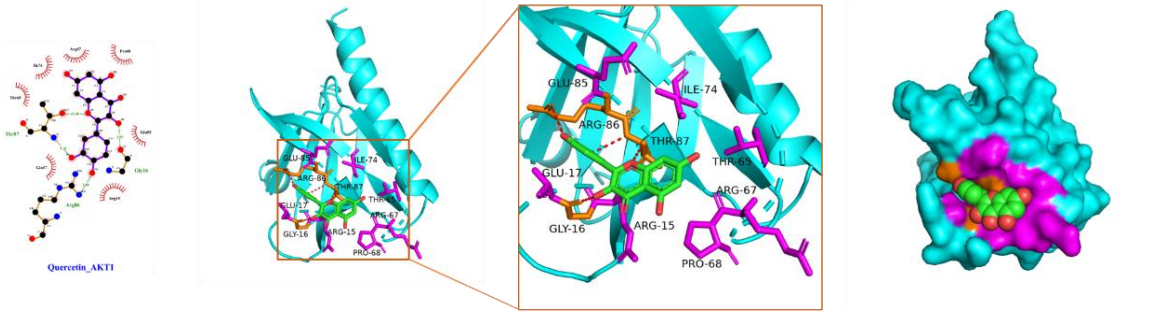
The schematic diagram of docking of core ingredients with important target protein molecules is shown in Figure 8.

Table 3 Information of molecular docking results.

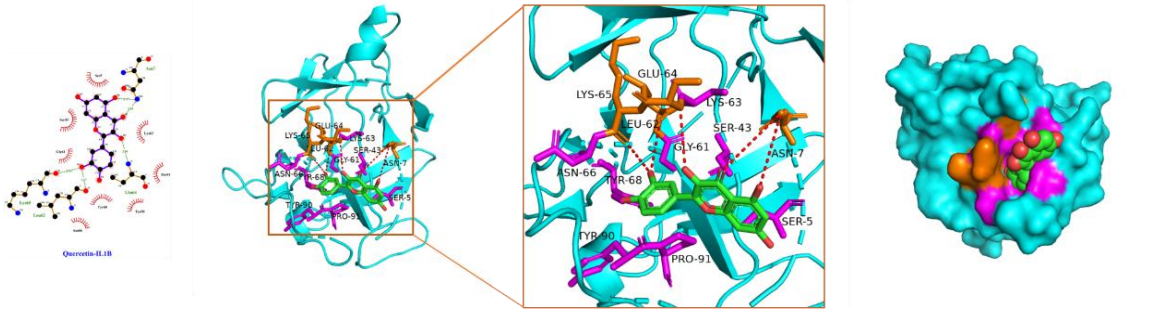
Protein and PDB ID	Name of compound	Binding energy score (kcal/mol)
AKT1(2UVM)	kaempferol	-6.2
AKT1(2UVM)	quercetin	-6.5
IL-1β(6Y8I)	quercetin	-6.8
IL-6(1ALU)	quercetin	-5.7



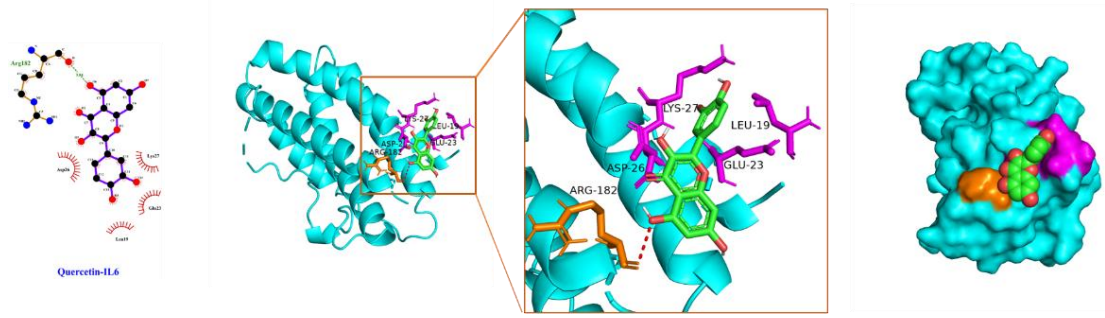
(A) Kaempferol and AKT1



(B) Quercetin and AKT1



(C) Quercetin and IL-1β



(D) Quercetin and IL-6

Figure 8 Schematic diagram of docking of core ingredients with important target protein molecules.

4 Discussion

PSD is the commonest and severest psychoneurologic complication after stroke, and has the morbidity and disability rate increasing year by year in recent years, impacting nearly one third of stroke patients [23]. The current treatment lacks effective specific methods and mainly depends on antidepressant drugs; however, long-term use of these drugs can lead to a series of problems such as adverse reactions and drug resistance. TCM has the advantages of multi-target, multi-system, multi-channel and small toxic and side effects in treatment of PSD [24].

The relevant research pointed out that Radix Bupleuri-Paeoniae Radix Alba is one of the top five drug pair used most frequently in the treatment of PSD [14]. However, the specific mechanism of active ingredients in Radix Bupleuri-Paeoniae Radix Alba remains undefined currently. This study performed analyses through network model construction, and screened out 29 active ingredients from Radix Bupleuri-Paeoniae Radix Alba and 145 potential targets for PSD treatment. By constructing a drug-active ingredients-potential target network and PPI network, the main ingredients of Radix Bupleuri-Paeoniae Radix Alba for treating PSD were predicted to be quercetin, kaempferol, and β -sitosterol. Among them, quercetin and kaempferol had the most corresponding targets, and the core targets of Radix Bupleuri-Paeoniae Radix Alba for treating PSD included AKT1, IL-6, IL-1β, etc. Previous study have found that Xiaoyao-jieyu-san also improves depression after stroke, and a large number of the active components include quercetin, kaempferol, etc. [25]. Quercetin possesses wide pharmacological effects, such as anti-oxidant, free radical scavenging, anti-inflammation, anti-tumor and neuroprotection [26], and has been reported to have good anti-depression effect in recent years [27]. Kaempferol has pharmacological activities such as antioxidant, anti-inflammation, and anti-tumor, and has been found through animal experiments to increase the levels of dopamine, NA, and 5-HT in the brain of depressed rats, thereby exerting antidepressant effects [28]. β-sitosterol is the main drug Incltains, study have found that β-sitosterol can manifest To increase the levels of 5-HT and NE in the brain of depressed mice depression-like behavior [29]. Consistent with the screened active ingredients in this study, we confirmed that quercetin and kaempferol may be one of the core effective materials of Radix Bupleuri-Paeoniae Radix Alba in treatment of PSD.

Further, our molecular docking experiments demonstrated quercetin and kaempferol interacted with key proteins, AKT1, IL-6 and IL-1β. Research has shown that IL-6 and IL-1β may play important roles in the occurrence and development of PSD, and inflammatory cytokines such as IL-6 and IL-1β can maintain high levels for several months after onset of stroke. This chronic immune inflammatory response can lead to mood changes associated with depression [30]. Chaihu-Shugan-San regulates the polarization of

microglia through the JAK/STAT3-GSK3 β /PTEN/Akt signaling pathway and subsequently inhibits neuroinflammation to ameliorate the symptoms of PSD. Meanwhile, Numerous studies have demonstrated that cytokine levels, such as IL-1β, IL-6, TNF-α, and IL-18, are signifcantly elevated in patients with PSD [31-33], suggesting that the presence of these cytokines in the brain may contribute to PSD development. Accordingly, the core proteins, AKT1, IL-6 and IL-1β, are instrumental in PSD, denoting that Radix Bupleuri-Paeoniae Radix Alba may exert anti-PSD activity by regulating these core proteins, and further *in vitro* experiments are needed to verify this hypothesis.

In addition, KEGG pathway analyses revealed that several signaling pathways were implicated in PSD treatment using Radix Bupleuri-Paeoniae Radix Alba, mainly including “lipids and atherosclerosis”, “chemical carcinogenesis-receptor activation”, “hepatitis B”, “AGE-RAGE signaling pathway in diabetes complications” and “Kaposi sarcoma associated herpesvirus infection”. According to a related report, atherosclerosis serves as one of the risk factors of PSD, and Dachaihu Tang containing “Radix Bupleuri-Paeoniae Radix Alba” can regulate blood fat, inhibit IL-1β expression and dampen the formation of atherosclerosis [34]. Moreover, emotions are closely related to the liver, with one-third of patients with hepatitis or cirrhosis experiencing depression. A key link between depression and liver disease seems to be the inflammatory process, in which increased microbiota and intestinal permeability play a crucial role [35]. While the findings of this study provide valuable insights into the potential mechanisms underlying the effects of Radix Bupleuri-Paeoniae Radix Alba in treating Post-Stroke Depression (PSD), it is essential to validate these results through rigorous animal experiments. Such studies are crucial for confirming the pharmacological effects of these compounds *in vivo*, assessing their bioavailability, and elucidating their mechanisms of action in a biological context.

5 Conclusion

Collectively, Radix Bupleuri-Paeoniae Radix Alba can mediate “lipids and atherosclerosis signaling pathway”, “hepatitis B” and other signaling pathways, and act on core targets including AKT_1, IL-6 and IL-1 β via quercetin and kaempferol, thereby treating PSD. This study preliminarily predicts the mechanism of Radix Bupleuri-Paeoniae Radix Alba in PSD treatment, providing a theoretical basis for further research on its underlying mechanisms. Our results provide a new research base and biomarker for PSD treatment to improve treatment efficacy. Integration of these bioactive compounds into treatment options may improve patient outcomes by addressing the psychological and physiological aspects of rehabilitation, potentially improving the quality of life of the affected individuals.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Y.F. and L.C. contributed to the conception and design of the paper. X.Z. and Z.Z. searched the literature and wrote the first draft of the manuscript. All the authors contributed to and approved the manuscript for submission.

Ethics Approval and Consent to Participate

The manuscript didn't involve any human or animal subjects, therefore no ethical approval was required for this article.

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Availability of Data and Materials

All data are available on request from the first author.

Supplementary Materials

Not applicable.

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