The Network Pharmacology Study And Molecular Docking To Investigate The Potential Mechanism of Acoritataninowii Rhizoma Against Alzheimer's Disease

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Abstract

Background and objective: Alzheimer's Disease (AD) is considered as a progressively developing neurodegenerative disease with an insidious onset that induces increased cost of social burden and decreased quality of life. Acoritataninowii Rhizoma produced the effects of resuscitating and eliminating phlegm, dispelling dampness and appetizing, refreshing mind and nourishing the mind, and exerted the activities of anti-dementia and improving learning and memory. while little was relevant to its anti-AD mechanism. The present study explored the potential mechanism of Acoritataninowii Rhizoma defend AD by network pharmacology and molecular docking.

Methods: The bioactive ingredients of Acoritataninowii Rhizoma were screened by absorption, distribution, metabolism as well as excretion evaluation and obtained from databases retrieval. Genes associated with AD or ingredients were searching by databases, and the overlapping genes between AD and ingredients were analyzed by the Venn diagram. Moreover, the network of Acoritataninowii Rhizoma-ingredients-targets-AD was visualized by cytoscape software. Furthermore, protein-protein interaction, gene ontology, pathway enrichment and molecular docking were conducted to evaluate potential factors of Acoritataninowii Rhizoma against AD.

Results: 4 potential compounds were considered as bioactive ingredients after screening ADME. 81 ingredients-related genes and 6765 AD-related genes were screened by databases with 61 overlapping genes. The bioactive ingredients derived from Acoritataninowii Rhizoma (e.g Cycloartenol, (1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan, 8-Isopentenyl-kaempferol, kaempferol) and target proteins (e.g AKT1, JUN, ESR1, CASP3, MAPK14, RELA) with high degree in the network were associated with in mitogen-activated protein kinase (MAPK) of DNA-binding transcription factor. Moreover, Acoritataninowii Rhizoma might play a significant in the treatment of AD which induced Fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, AGE-RAGE signaling pathway in diabetic complications.

Conclusion: The bioactive ingredients and potential mechnism of Acoritataninowii Rhizoma defended AD was analyzed by network pharmacology and molecular docking. This study provided a research basis and scientific evidence for supporting the activities of Acoritataninowii Rhizoma against AD.

Introduction

Alzheimer's disease (AD) is an insidious and progressive neurodegenerative disease. Clinically, comprehensive dementia is characterized by memory impairment, aphasia, apraxia, agnosia, impairment of visual spatial skills, executive dysfunction, and personality and behavior changes. It affects the physical health seriously and quality of life of the elderly. It is very complex that pathogenesis has no specific medicine at present, and the disease usually lasts for 10 to 25 years. The chronic nature of AD, as well as its attacks on the protective cognitive vulnerability, places a huge emotional and financial burden on patients, their families and society as a whole. On the point of view in the harm of Alzheimer's disease, effective prevention of Alzheimer's disease is essential for the benefit of patients.

At present, the main medicinal treatments used in the prevention of AD include acetylcholine inhibitors, glutamate receptor antagonists, Piracetam and other drugs that could alleviated the symptoms of AD. Tacrine produced serious side effects that affect the digestive function and has been discontinued. Galantamine and Rivastigmine caused indigestion, and Memantine caused dizziness, headache and other adverse reactions⁹. Donepezil was only suitable for patients with mild and moderate AD. The drugs mentioned above for the treatment of Alzheimer's disease (AD) represent our current best drug treatments, but their average overall effect was relatively slight with change the course of underlying neurodegenerative process.

In addition to the medicinal treatments mentioned above, which are well studied at the present stage, there are many other therapeutic drugs. The most typical one is the application of Traditional Chinese medicine to Alzheimer's disease. Compared with medicinal treatments, Traditional Chinese medicine unique to China is favored because of its advantages of effecton and less side effects. In recent years, the application of traditional Chinese medicine extract and its derivatives in the treatment of AD has been increasing.

Acoritataninowii Rhizoma is a well-known herb that has been used in traditional Chinese medicine (TCM) for a long time and kept track in the Shennongji Classic of Materia Medica (listed as top grade) and present in much of Chinese traditional prescriptions for thousands of years. Acoritataninowii Rhizoma is commonly used as Fangxiangkaiqiao Chinese medicine, mainly containing volatile oils, flavonoids, anthraquinones, alkaloids, triterpenoid saponins, phenylpropanoids, organic acids, amino acids and glucose. It exerted pharmacological effect of sedative, anti-depressive, anti-dementia, cardiomyopathy protection, stomachic, anti-oxidation, relieving cough and asthma, anti-inflammatory and anti-fatigue, etc. It contained in the main constituent drug in the classic traditional Chinese prescription for the treatment of central nervous system diseases. It also showed superior pharmacological activity in the cardiovascular system, respiratory system and digestive system. Some scholars have done relevant researches on AD, so it is of great significance to further explore the mechanism of action of Acoritataninowii Rhizoma against AD via data mining.

Traditional Chinese medicine (TCM) uses multiple ingredients and targets to prevent disease, rather than a single whole. The holistic view of network pharmacology is consistent with that of TCM. Network pharmacology is a widely used bioinformatics method to analyze the underlying mechanism between drugs and diseases. In recent years, network pharmacology has been used to predict the potential targets and pathways of certain components in various diseases, even in mental diseases that were closely related to signal transduction. In addition, biological information resources such as molecular docking have also been developed, providing more evidence for rapid system analysis. Through network pharmacological analysis and prediction, molecular docking is designed to evaluate the binding potential of protein and ligand.

Therefore, this study introduced a pharmacological network combined with molecular docking methods to evaluate the effects of Acoritataninowii Rhizoma on AD and potential molecular mechanisms. It is supposed to provide the feasibility of Acoritataninowii Rhizoma for the therapy of AD and reliable information. This research was conducted in the following steps: (1) the ingredients of Acoritataninowii Rhizoma and its corresponding targets and AD-related

targets were retrieved from databases; (2) The network of medicine-ingredients-targets-disease was constructed, and performed a visual analysis; (3) Through network analysis and construction of key components, core targets, top-level functions and signal pathways; (4) By docking with the top target and the corresponding components of the molecule, the potential target is obtained and verified. The schedule was presented in Fig. 1.

Materials And Methods

Collection and screening of active ingredients in Acoritataninowii Rhizoma

The collected ingredients of Acoritataninowii Rhizoma were obtained from Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM) (<http://bionet.ncpsb.org/batman-tcm/>) and Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (<https://tcmssp.com/tcmssp.php>), Traditional Chinese Medicines Integrated Database (TCMID) (<http://www.megabionet.org/tcmid/>). Before predicting the target, absorption, distribution, metabolism, and excretion (ADME) was employed to screen the biologically active ingredients that produce therapeutic effects, and eliminate ingredients with inferior pharmacological activity. In order to obtain ingredients with oral absorption, utilization and biological properties for further analysis, the candidate ingredients meet molecular weight (MW) ≤ 500 , oral bioavailability (OB) $\geq 30\%$, and drug-likeness (DL) ≥ 0.18 three parameters. Collected targets was based on the screened biologically active ingredients. Target gene names were standardized from uniprot database (<http://www.uniprot.org/uploadlists/>). After collection and screening, Cytoscape3.7.2 software was utilized to construct the network of medicine-ingredients-targets.

Genes collection for AD

The information of AD-related target genes was gathered from the previously studied databases¹¹, including PharmGKB (<https://www.pharmgkb.org/>), online mendelian inheritance in man database (OMIM) (<https://omim.org/>), disgenet database (<https://www.disgenet.org/>), therapeutic targets database (TTD) (<http://db.idrblab.net/ttd/>), DrugBank database (<https://www.drugbank.ca/>), GeneCards (<https://www.genecards.org/>). Only the "Homo sapiens" proteins associated with AD were selected. Gene names were standardized in uniprot database^{11,17}.

Intersection of targets between active ingredients and disease

According to the collected genes of Acoritataninowii Rhizoma and AD, the cross targets between biologically active ingredients and disease were obtained, so as to predict the possibility of Acoritataninowii Rhizoma's pharmacological effects on AD. The crosses was processed by network analysis software Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>) and was shown as a Venn diagram and the number of intersecting genes is automatically calculated.

The network of medicine-ingredients-targets-disease construction

On the basis of the intersection of biologically active ingredients and disease targets, Cytoscape3.7.2 software was used to construct a drug-component-target-disease network, and the interaction between Acoritataninowii Rhizoma-ingredients-targets-AD was speculated. After the visual network is constructed, topological properties such as "Degree" and "Betweenness Centrality" were calculated through the "Network Analyze" in the software.

Protein-protein interaction (PPI) network analysis

Learn more about the cross-target intersection between the biologically active ingredients and diseases. The functional protein association network was completed by Cytoscape3.7.2 software and STRING database (<https://string-db.org/>), which selection condition was "Homo sapiens". And set the confidence score of the correlation degree ≥ 0.400 (medium confidence) as the cut-off value to obtain a network. In addition, the counts of interactions between the proteins were also calculated.

Gene ontology (GO) enrichment analysis

GO enrichment analysis is a commonly used computational model to provide a logical framework for gene functions and annotations. The current study uses R package cluster Profiler to perform GO enrichment analysis under the "Homo sapiens" setting, and sets the threshold value of $p < 0.05$. Changes in genetic functions were manifested in molecular function (genes that regulate molecular activity), cellular components (the relationship between gene function and cellular structure), and biological process (biological procedures that organisms achieve through genetic programs). Furthermore, the number of genes in each GO were computed to show the potential genetic function of Acoritataninowii Rhizoma affecting AD, and the network of Go enrichment was constructed by Cytoscape3.7.2 software.

Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The KEGG analysis provided information for the possible pathological pathways of AD, and based on the above information, participation of Acoritataninowii Rhizoma pharmacological pathway was preliminarily speculated. R package clusterProfiler was also used for KEGG enrichment analysis, and used to merge KEGG pathways to construct a functionally efficient pathway network. The enrichment was performed in the conditions of "Homo sapiens", a threshold value of $p < 0.05$. Cytoscape3.7.2 software was utilized to calculate the number of genes in each pathway to construct a network of target pathways. In addition, a map of the main influences path was also collected.

Molecular docking

In network pharmacological research, Molecular docking was used to verify ingredient-target association^{8,9,23}. The molecular structure of each ingredient in the work was obtained from TCMSP database (<https://tcmssp.com/tcmssp.php>) and saved as a mol2 format file. Download the 3D structures of the target protein from the RCSB PDB database (<http://www.rcsb.org/>) and save it as a PDB format file. Before the docking process, the ligand and protein were prepared in the autodock software. The crystal structure of target proteins was preprocessed, including the removal of water molecules, protonation

hydrogenation, and Gasteiger charges calculation. The minimal energy conformation requires the structure of the ligand. Protein ligand with the lowest binding energy is considered the most likely target.

Results

Potential targets in Acoritataninowii Rhizoma and AD

Through searching TCMSP, TCMID and BATMAN-TCM, detailed information of 4 active ingredients with 81 target genes in Acoritataninowii Rhizoma was obtained, as shown in Table 1. In addition, the topological properties of the network construction based on the Acoritataninowii Rhizoma-ingredients-targets show that the number of nodes is 85 and the number of edges is 106 (Fig. 2). The network centralization is 0.738 and the network density is 0.029. The ingredient and target with maximum degree are kaempferol (64) and NCOA2 (3) [PGH2(3)] [CALM1(3)], respectively. By searching database such as DrugBank, GeneCards, OMIM, DisGeNET, TTD and PharmGKB, a total of 6765 target genes were found to be AD-specific target genes. The result show that the pharmacological characteristics of Acoritataninowii Rhizoma involve a variety of biologically active ingredients and target genes. Similarly, the pathology of AD is also related to many target genes.

The potential targets intersection

As shown in Fig. 3A, the ingredient-related genes of Acoritataninowii Rhizoma were matched with AD-related genes through the Venn diagram, and 61 overlapping genes were calculated, including CYP3A4, GABRA1, GABRA2, ADRA1B, CYP1A2, ADRA1D, KCNH2, NOS3, AKT1, PPARG, HMOX1, NOS2, INSR, GSK3B, RELA, CASP3, CALM1, PPP3CA, ESR1, MAPK14, MAPK8, JUN, XDH, CDK1, F2, BAX, ICAM1, MMP1, SLC6A2, AR, STAT1, GSTM1, BCL2, NR3C2, IKBKB, DPP4, PRSS1, VCAM1, GSTP1, PRKACA, AHR, CYP1A1, KCNMA1, CDK2, PGR, SLC2A4, SLPI, PIK3CG, NR1I2, RXRA, CHEK1, PSMD3, CCNA2, HAS2, HSP90AB1, PIM1, TOP2B, AHS1, DIO1, PYGM, SCN5A. Overlapping genes accounted for 0.9% of the total genes, indicating that the influence of Acoritataninowii Rhizoma on AD was possibly associated with above-mentioned multiple overlapping genes.

Network of medicine-ingredients-targets-disease construction

As is indicated in Fig. 3B and table 2, the network construction of medicine-ingredients-targets-disease including 67 nodes and 140 edges. In the Venn diagram, the network contains 4 biologically active ingredients and 61 overlapping genes. The cytoscape3.7.2 software analyze other critical parameters, such as network density (0.063), network centralization (0.888), average number of neighbors (4.149), network heterogeneity (2.218). The pharmacological effects of Acoritataninowii Rhizoma on AD were carried out through active ingredients and targets genes as indicated in mapping.

Network of PPI

Figure 4B - a PPI network was constructed by STRING because provided 61 overlapping proteins and that analyze their interaction that looks like this. 59 proteins except two: HAS2, DIO1, which had been established the connection. The main parameters of the network were analyzed through the STRING database: the number of nodes (61), the number of edges (355), the average node degree (11.6), and the average local clustering coefficient (0.555). Additionally, the degree of each node was constructed by GraphPad Prism 5.0 (version 2.0; GraphPad Software Inc., San Diego, CA) (Fig. 4A). Top 10 protein node degrees were AKT1, JUN, CASP3, AR, ESR1, MAPK8, RELA, MAPK14, NOS3, HSP90AB1. The results shown that the pharmacological effects of active ingredients of Acoritataninowii Rhizoma on AD might be associated with the interaction of these core proteins.

GO enrichment analysis for target genes

The GO enrichment analysis further evaluates the functions of overlapping target genes from three aspects: molecular function, biological process and cellular components. Threshold value based on $p < 0.05$ and from the above three aspects, it was constructed that GO enrichment was in the top 30. The molecular function results indicated that the top 5 (generatio) term were protein serine/threonine kinase activity, ubiquitin-like protein ligase binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin protein ligase binding and DNA-binding transcription factor binding (Fig. 5A and table 3). In addition, the number of nodes is 59 and the number of edges is 122 was illustrated in Fig. 5B, which was the topological properties of network construction. More meaningful parameters were tested, such as network centralization (0.123), network heterogeneity (0.581), average number of neighbors (4.136), network density (0.071). The molecular function and target gene with maximum degree were GO:0004674 (11) and ESR1 (8), respectively.

Top 5 (generatio) in the Biological process were response to metal ion, response to lipopolysaccharide, response to molecule of bacterial origin, cellular response to chemical stress and response to oxygen levels (Fig. 6A and table 4). Moreover, from the topological properties of network construction (Fig. 6B), it could be known that the number of nodes is 65 and the number of edges is 256. Other meaningful parameters were further tested, such as network density (0.123), network centralization (0.131), average number of neighbors (7.877), network heterogeneity (0.602). The biological process and target gene with maximum degree were GO:0010038 (15) and ICAM1 (16), respectively.

As shown in Fig. 7A and table 5, membrane raft, membrane microdomain, membrane region, plasma membrane raft and ion channel complex were the top 5 (generatio) of cellular components. Moreover, from the topological properties of network construction (Fig. 7B), it could be known that the number of nodes is 57 and the number of edges is 131. Other meaningful parameters were further tested, such as network density (0.082), network centralization (0.174), average number of neighbors (4.596), network heterogeneity (0.690). The cellular components and target gene with maximum degree were GO:0045121 (14) and SCN5A (10), respectively. The results showed that the pharmacological effects of bioactive ingredients of Acoritataninowii Rhizoma on were closely linked to MAPK-coupled receptor activity or signal pathways. In addition, the transcription factors have a significant impact on signal transduction.

KEGG enrichment analysis for target genes and mapping

Table 6 and Fig. 8A - KEGG results showed that 61 overlapping genes in 20 signaling pathways were significantly enriched, as showed here. Top 5 (generatio) signaling pathways were Fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, AGE-RAGE signaling pathway in diabetic complications and Human immunodeficiency virus 1 infection. In addition, the number of nodes is 57 and the number of edges is 218 were illustrated in Fig. 8B, which was the topological properties of network construction. Other parameters including network density (0.137), network centralization (0.210), average number of neighbors (7.649), network heterogeneity (0.691) were tested, also. The signaling pathway and target protein with maximum degree were hsa05418 (15) and RELA (19), respectively. Top 3 mapping signaling pathways were Fluid shear stress and atherosclerosis (Fig. 9), Kaposi sarcoma-associated herpesvirus infection Fig. 10) and Epstein-Barr virus infection (Fig. 11), indicating that these signaling pathways play an important role in the pharmacological effects of bioactive ingredients originate from *Acoritataninowii Rhizoma* on AD.

Molecular docking between ingredients and target proteins

As shown in Table 7, 5 target proteins (AKT1, CASP3, ESR1, JUN and MAPK14) and 4 bioactive ingredients (MOL000422/kaempferol, MOL003542/8-Isopentenyl-kaempferol, MOL003576/(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan, MOL003578/Cycloartenol) were selected to conduct molecular docking based on their high degree in the network of *Acoritataninowii Rhizoma*- ingredients-targets-AD. The lower the binding energy, the higher the binding affinity. The binding energy range of each target protein was as followed: AKT1 (-6.04 to -9.07 kcal/mol), CASP3 (-6.14 to -7.9 kcal/mol), ESR1 (-5.65 to -10.85 kcal/mol), JUN (-6.31 to -8.98) and MAPK14 (-6.63 to -9.29 kcal/mol). Additionally, the highest binding affinity of each biologically active ingredient was as followed: MOL00042 (ESR1: -6.92 kcal/mol), MOL003542 (CASP3: -6.96 kcal/mol), MOL003576 (CASP3: -7.9 kcal/mol), and MOL003578 (ESR1: -10.85 kcal/mol). The results show that these bioactive ingredients and core target proteins were of great significance to the possible mechanism of *Acoritataninowii Rhizoma* defensive against AD. Moreover, ESR1 and MAPK14 were shown higher affinity for these biologically active ingredients.

Discussion

TCM is a complicated system with multiple components and multiple targets, and each ingredient produces a synergistic effect. The composition of TCM is complex, it is difficult to study its effect from the perspective of mixture, and it is also hard to explain its mechanism from the perspective of modern medicine. Network Pharmacology supplied new perspectives for research on complex TCM system. The pharmacology of traditional Chinese medicine system has established a model of the interaction of drug molecules, protein targets, tissues, organs and other elements to clarify and predict the efficacy and toxicity of traditional Chinese medicine. This study evaluated the possibility of *Acoritataninowii Rhizoma*'s pharmacological characteristics in the treatment of AD with network pharmacology study and molecular docking. In order to explore the multi-ingredients and multi-targets research mode of TCM, network pharmacology research provided an innovative way for the study of traditional Chinese medicine ingredients. The results showed that the biologically active components and target proteins with prominent nodes occupy a pivotal position in the network and play an important role in the pharmacological effects of *Acoritataninowii Rhizoma*.

Acoritataninowii Rhizoma not only has the functions recorded in the Pharmacopoeia of the People's Republic of China, but also has regulatory and therapeutic effects on the nervous system, cardiovascular system, immune system, and reproductive system, while little was known about its mechanism of action on AD. Through the search of the database and the screening of MW, OB, DL, it was determined that these ingredients were four biologically active in *Acoritataninowii Rhizoma*. The genes accounted for 0.9% in total were shown in medicine-ingredients-targets-disease interaction network. It was indicated that might be at the core genes for the pharmacological profile of *Acoritataninowii Rhizoma* suggesting the effects of *Acoritataninowii Rhizoma* on AD was possibly associated with multiple overlapping genes.

Active ingredients

Among the 4 bioactive ingredients of *Acoritataninowii Rhizoma* have been passed the ADME screening. It is a natural flavonol present in different plant species, Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), which has been described to possess potent anti-inflammatory properties. Some epidemiological studies have found a positive association between consumption of food containing kaempferol and a reduced risk of developing several disorders such as cancer and cardiovascular diseases. Numerous preclinical studies have shown that kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, anti-osteoporotic, estrogenic/antiestrogenic, anxiolytic, analgesic and anti allergic activities. Beg et al. found that Kaempferol could be used as a possible drug to fight the development of Alzheimer's disease through animal experiments, Exposure to Kaempferol in AD patients could delay climbing ability, memory loss, reduce oxidative stress and acetylcholinesterase activity. Among the most promising group of substances with potential activity against AD are the flavonoids, including myricetin, morin, rutin, quercetin, fisetin, kaempferol, apigenin and glycerin, which have been shown, in vitro, to possess anti-amyloidogenic and fibril-destabilization activity, as well as being able to act as metal chelators and to suppressing oxidative stress. Cycloartenol is a plant sterol compounds, is made up of 2,3 - oxidized squalene (OS) in cycloartenol synthase (CAS) catalyzed by cycloartenol synthase, and biosynthesis of many kinds of one of the key precursor substance of sterol compounds, exert many pharmacological activities, such as anti-inflammatory, anti-tumor, antioxidant, biological activity and resistance against alzheimer's disease²⁹. Jung HA et al. evaluated the anti-Alzheimer's disease effect of Cycloartenol by inhibition of AChE, BChE and beta amyloid precursor protein (APP) lyase 1 (BACE1), suggesting Cycloartenol may play a major role as AChE inhibitor against Alzheimer's disease. To sum up, the above three active ingredients have anti-inflammatory, anti-oxidant and anti-dementia effects.

PPI network and proteins

Not only the overlapping genes were investigated, the interaction among the overlapping proteins was also explored. 59 shared target proteins regulated by *Acoritataninowii Rhizoma* and AD, including AKT1, JUN, CASP3, ESR1, AR, MAPK8, RELA, NOS3, MAPK14 were the key target proteins with high node degree by analyzing PPI network. Through experiments in APP/PS1 mice, Ahmad F et al. proved that Akt1 oxidative modification mediated by reactive oxygen ROS led to synaptic dysfunction in AD, which was considered to be the loss of activity-dependent protein translation that was crucial for synaptic plasticity and

maintenance. Liu SY reported that Alzheimer's disease (AD) was closely related to type 2 diabetes mellitus (T2D), in which V-Akt murine thymoma viral oncogene homologue 1 (AKT1) play an important role in protein synthesis pathway and cell apoptosis. The interaction among the JUN, CASP3, ESR1 in PPI network indicated the proteins interacted each other were involved in the pharmacological profile of Acoritataninowii Rhizoma and pathologists of AD.

CASP (cysteinyI aspartate specific proteinases) is the specific mediator and executor of apoptosis. Caspase-3 not only reflects the level of apoptosis, but also indicates the existence of apoptosis factors. Caspase-3 is an important factor of apoptosis and a common pathway of apoptosis signal transmission. Animal experiments conducted by Mao et al. showed that the expression of CASP3 protein in hippocampus CA1 region of rats in the VD group increased with time, and the over expression of CASP3 protein could lead to the apoptosis of neurons in hippocampus CA1 region of rats. MAPK8 (mitogen-activated protein kinase 8) is a group of protein kinases that can be stimulated by different extracellular processes and is mainly concentrated in the TNF signaling pathway. The AR (androgen receptor) plays an important role in the pathogenesis of castration-resistant prostate cancer, involving gene amplification and mutation, regulatory factor changes, apoptosis resistance, tumor immunity and so on.

GO

On the basis of GO functional analysis, it was evaluated that in molecular function, biological process and cellular components, the common target genes were involved in more than 30 signal transduction functions. The following functions were of significance to the pharmacological effects of Acoritataninowii Rhizoma on AD: molecular function and biological process were associated with several receptors or signal pathways, like lipopolysaccharide, metal ion, protein serine/threonine kinase, which were relevant to MAPK. The functions/process were correlated with the synaptic or postsynaptic membrane in the cells of the following target genes including AKT1, EAR1, JUN, CASP3, PPARG, CDK1, BCL2. Cellular oxidative stress generates DNA damage and activates the important mitogen-activated protein kinase 14 (p38MAPK) that is involved in pathology like Alzheimer's disease. mitogen-activated protein kinase (MAPK) leads to increased production of proinflammatory cytokines and neurodegeneration. Mitogen-activated protein kinase 14 (MAPK14), MiR-22-3p overexpression reduced A β deposit and alleviated AD symptoms by targeting and regulating MAPK14 expression, which ameliorated AD symptoms. Secondly, it may decrease tau protein phosphorylation through down-regulating the phosphorylation level of MAPK14. Brain p38 α mitogen-activated protein kinase (MAPK), a potential therapeutic target for cognitive dysfunction based on the neuroinflammation-synaptic dysfunction cycle of pathophysiology progression, provides an innovative pharmacological strategy via inhibiting the same activated target in both glia and neurons, thereby enhancing the possibility for efficacy. The highly selective, brain-penetrant p38 α MAPK inhibitor MW150 attenuates cognitive dysfunction in two distinct Alzheimer's disease (AD)-relevant models and avoids the problems encountered with previous mixed-kinase inhibitor drug candidates.

AP-1 (activating protein-1) is a collective term referring to dimeric transcription factors composed of Jun, Fos or ATF (activating transcription factor) subunits that bind to a common DNA site, the AP-1-binding site. It blocked that the activation of nuclear factor (NF)- κ B and mitogen-activated protein kinases (MAPKs) e.g., JNK (mitogen-activated protein kinase 8) and p38 MAPK, thereby attenuated the nuclear translocation of NF- κ B subunit, p65, and activator protein-1 (AP-1) proteins (c-Fos and c-Jun). activation of p38MAPK and JNK, resulting in the increased production of NO. activate the NF- κ B, p38MAPK and JNK pathways to induce a prolonged microglial activation which may downregulate the neuroprotective events in the brain of neurodegenerative diseases. In addition, Zhu T et al. suggested that NMB-induced COX-2 and IL-6 expression were mediated via p65 and c-Jun activation. Thus, MAPK play an important role in three biological functions.

KEGG

Furthermore, the possible pathways of Acoritataninowii Rhizoma on AD were analyzed by KEGG enrichment. The results indicated that pharmacological effects of Acoritataninowii Rhizoma involve multiple signaling pathways, like Fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, AGE-RAGE signaling pathway in diabetic complications, Human immunodeficiency virus 1 infection, Hepatitis B, Human T-cell leukemia virus 1 infection, IL-17 signaling pathway, TNF signaling pathway. The Fluid shear stress and atherosclerosis of signal pathway induces the expression of Fractalkine mRNA in endothelial cells to increase, which makes blood vessels harden to produce plaques, which in turn affects hemodynamics and causes cardiovascular and cerebrovascular diseases. The AGE-RAGE (advanced glycosylation end product- receptor of AGE) signaling pathway, which RAGE acts as a pro-inflammatory mediator, AGEs is an important ligand of RAGE and it can activate RAGE receptors, activate the transcription factor NF- κ B, cause inflammation and oxidative stress, damage the structure and function of brain neurons and hippocampus, and lead to decreased learning and memory functions. TNF- α (Tumor Necrosis Factor- α) is a pro-inflammatory cytokine, which participates in cell differentiation, apoptosis and induce inflammation through up-regulation of signalling pathways such as MAPK and NF- κ B. The signal pathway composed of PI3K and AKT is an anti-apoptotic and pro-survival signal pathway. PI3K/AKT pathway has been shown to play a critical role on the neuroprotection and inhibiting apoptosis via enhancing expression of the SODs. This pathway appears to be crucial in Alzheimer's disease because it is related to the tau protein hyper-phosphorylation. A number of studies showed that Chinese medicine play an anti-AD effect by regulating the PI3K/AKT signaling pathway.

Molecular Docking

Moreover, this study also proposes network pharmacology, molecular docking and other widely used research methods for protein-ligand interactions, supplementing the pharmacological effects of Acoritataninowii Rhizoma on AD. Based on their high degree in the network of Acoritataninowii Rhizoma-ingredients-targets-AD, Several target genes (ESR1, PTSG2, NOS2, and ADRB2) and bioactive ingredients (MOL002565, MOL003896, MOL004891, MOL000392 and MOL000497) were selected to conduct molecular docking. The docking results indicated that accord with the network, these biologically active ingredients and core target genes might be the potential key to the pharmacological effects of Acoritataninowii Rhizoma on AD. NOS2 and ESR1 were shown high affinity for these active ingredients. Combining with the analysis results of network pharmacology study, it seems that the pharmacological effects of biologically active ingredients (MOL002565 and MOL004891) originated from Acoritataninowii Rhizoma on AD were related to NOS2 and ESR1, respectively. However, the docking results need to be confirmed via more studies.

Conclusion

Above all, the pathogenesis of AD is complex, with signalling pathways intersecting and regulating each other, presenting a multi-target and multi-pathway model. The active ingredients of *Acoritataninowii* Rhizoma could regulate target genes/proteins that were related to signal pathways, play a role in stabilizing the signal pathway, and reveal the mode of action of multi-ingredient-multi-target-multi-pathway in TCM. Further analysis of the pharmacological effects of *Acoritataninowii* Rhizoma on AD, as well as the targets and signal pathways that interact with active ingredients still need further verification.

Declarations

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Tables

Table 1 Information of ingredients from Acoritataninowii Rhizoma screening by databases

Mol ID	Ingredient	MW	OB (%)	DL
MOL003578	Cycloartenol	426.8	38.69	0.78
MOL003576	(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan	386.48	52.35	0.62
MOL003542	8-Isopentenyl-kaempferol	354.38	38.04	0.39
MOL000422	kaempferol	286.25	41.88	0.24

Table2 Topological analysis based on network of medicine-ingredients-targets-disease

Node	Degree	BC	Node	Degree	BC	Node	Degree	BC
AD	61	0.61065	HAS2	2	0.00015	MMP1	2	0.00015
MOL000422	48	0.27964	CCNA2	2	0.00060	ICAM1	2	0.00015
MOL003542	23	0.06647	PSMD3	2	0.00015	BAX	2	0.00015
MOL003576	6	0.00442	CHEK1	2	0.00060	CDK1	2	0.00015
CALM1	4	0.00817	RXRA	2	0.00060	XDH	2	0.00015
sweetflag	4	0.02054	NR1I2	2	0.00015	JUN	2	0.00015
SCN5A	3	0.00480	PIK3CG	2	0.00015	MAPK8	2	0.00015
TOP2B	3	0.00140	SLPI	2	0.00015	MAPK14	2	0.00060
HSP90AB1	3	0.00140	SLC2A4	2	0.00015	ESR1	2	0.00060
PRSS1	3	0.00140	PGR	2	0.00015	CASP3	2	0.00015
DPP4	3	0.00140	CDK2	2	0.00060	RELA	2	0.00015
AR	3	0.00140	KCNMA1	2	0.00060	GSK3B	2	0.00060
F2	3	0.00140	CYP1A1	2	0.00015	INSR	2	0.00015
NOS2	3	0.00015	AHR	2	0.00015	HMOX1	2	0.00015
PPARG	3	0.00140	PRKACA	2	0.00015	AKT1	2	0.00015
KCNH2	3	0.00480	GSTP1	2	0.00015	NOS3	2	0.00015
ADRA1B	3	0.00590	VCAM1	2	0.00015	ADRA1D	2	0.00318
GABRA2	3	0.00140	IKBKB	2	0.00015	CYP1A2	2	0.00015
GABRA1	3	0.00140	NR3C2	2	0.01365	CYP3A4	2	0.00015
PYGM	2	0.00060	BCL2	2	0.00015	MOL003578	2	0.00059
DIO1	2	0.00015	GSTM1	2	0.00015	PPP3CA	1	0.00000
AHSA1	2	0.00015	STAT1	2	0.00015			
PIM1	2	0.00060	SLC6A2	2	0.00015			

Table 3 Molecular function of GO analysis

ID	Molecular function	P value	Count	Gene name
GO:0004879	nuclear receptor activity	2.29E-10	7	PPARG/ESR1/AR/AHR/PGR/NR1I2/RXRA
GO:0098531	ligand-activated transcription factor activity	2.29E-10	7	PPARG/ESR1/AR/AHR/PGR/NR1I2/RXRA
GO:0003707	steroid hormone receptor activity	8.24E-10	7	PPARG/ESR1/AR/NR3C2/PGR/NR1I2/RXRA
GO:0004674	protein serine/threonine kinase activity	2.65E-07	11	AKT1/GSK3B/MAPK14/MAPK8/CDK1/IKBKB/PRKACA/CDK2/PIK3CG/CHEK1/PIM1
GO:0044389	ubiquitin-like protein ligase binding	1.02E-06	9	KCNH2/GSK3B/RELA/JUN/STAT1/BCL2/PRKACA/HSP90AB1/SCN5A
GO:0031072	heat shock protein binding	3.34E-06	6	CDK1/BAX/AHR/CYP1A1/HSP90AB1/AHSA1
GO:0061629	RNA polymerase II-specific DNA-binding transcription factor binding	4.68E-06	8	PPARG/GSK3B/RELA/ESR1/MAPK14/JUN/STAT1/RXRA
GO:0031625	ubiquitin protein ligase binding	6.55E-06	8	KCNH2/GSK3B/RELA/JUN/BCL2/PRKACA/HSP90AB1/SCN5A
GO:0020037	heme binding	6.94E-06	6	CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A1
GO:0046906	tetrapyrrole binding	1.05E-05	6	CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A1
GO:0016725	oxidoreductase activity, acting on CH or CH2 groups	1.09E-05	3	CYP3A4/CYP1A2/XDH
GO:0016705	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	1.77E-05	6	CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A1
GO:0005496	steroid binding	1.88E-05	5	CYP3A4/ESR1/AR/NR3C2/PGR
GO:0004497	monooxygenase activity	2.30E-05	5	CYP3A4/CYP1A2/NOS3/NOS2/CYP1A1
GO:0035173	histone kinase activity	2.56E-05	3	CDK1/CDK2/CHEK1
GO:0140297	DNA-binding transcription factor binding	2.61E-05	8	PPARG/GSK3B/RELA/ESR1/MAPK14/JUN/STAT1/RXRA
GO:0001091	RNA polymerase II general transcription initiation factor binding	4.26E-05	3	ESR1/AR/AHR
GO:0097110	scaffold protein binding	5.05E-05	4	KCNH2/NOS3/IKBKB/SCN5A
GO:0001223	transcription coactivator binding	6.57E-05	3	RELA/ESR1/AHR
GO:0042277	peptide binding	6.73E-05	7	PPARG/INSR/PPP3CA/GSTM1/GSTP1/RXRA/HSP90AB1
GO:0019903	protein phosphatase binding	0.000120241	5	AKT1/PPARG/MAPK14/STAT1/BCL2
GO:0097472	cyclin-dependent protein kinase activity	0.000148077	3	CDK1/CDK2/CCNA2
GO:0051721	protein phosphatase 2A binding	0.000180016	3	AKT1/STAT1/BCL2
GO:0016712	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	0.000180016	3	CYP3A4/CYP1A2/CYP1A1
GO:0033218	amide binding	0.000215674	7	PPARG/INSR/PPP3CA/GSTM1/GSTP1/RXRA/HSP90AB1

GO:0008395	steroid hydroxylase activity	0.000301701	3	CYP3A4/CYP1A2/CYP1A1
GO:0016709	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen	0.000326043	3	NOS3/NOS2/CYP1A1
GO:0051879	Hsp90 protein binding	0.000378447	3	AHR/CYP1A1/AHSA1
GO:0030544	Hsp70 protein binding	0.000406561	3	CDK1/BAX/CYP1A1
GO:0140296	general transcription initiation factor binding	0.000406561	3	ESR1/AR/AHR

Table 4 Biological process of GO analysis

ID	Biological process	P value	Count	Gene name
GO:0000302	response to reactive oxygen species	4.66E-13	13	NOS3/AKT1/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/STAT1/BCL2/GSTP1/CDK2/CCNA2
GO:0010038	response to metal ion	4.85E-13	15	CYP1A2/AKT1/HMOX1/CASP3/CALM1/PPP3CA/MAPK8/JUN/CDK1/ICAM1/BCL2/VCAM1/CYP1A
GO:0032496	response to lipopolysaccharide	2.35E-12	14	CYP1A2/NOS3/AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/VCAM1/GSTP1/CYP1A
GO:0002237	response to molecule of bacterial origin	3.96E-12	14	CYP1A2/NOS3/AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/VCAM1/GSTP1/CYP1A
GO:0062197	cellular response to chemical stress	5.19E-12	14	NOS3/AKT1/PPARG/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/BCL2/GSTP1/CDK2/SLC2A4/CC
GO:0072593	reactive oxygen species metabolic process	6.07E-12	13	CYP1A2/NOS3/AKT1/NOS2/INSR/MAPK14/XDH/F2/ICAM1/BCL2/GSTP1/CYP1A1/HSP90AB1
GO:0009410	response to xenobiotic stimulus	8.61E-12	13	CYP3A4/CYP1A2/PPARG/RELA/PPP3CA/ICAM1/GSTM1/GSTP1/AHR/CYP1A1/NR1I2/CCNA2/H
GO:0070482	response to oxygen levels	2.53E-11	14	AKT1/PPARG/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/KCNMA1/SLC2A4/PSI
GO:0046677	response to antibiotic	3.55E-11	13	HMOX1/RELA/CASP3/JUN/CDK1/ICAM1/STAT1/BCL2/VCAM1/GSTP1/AHR/CYP1A1/SLC2A4
GO:0048608	reproductive structure development	8.29E-11	14	NOS3/AKT1/PPARG/INSR/CASP3/ESR1/MAPK14/BAX/ICAM1/AR/BCL2/PGR/RXRA/HSP90AB1
GO:0061458	reproductive system development	9.09E-11	14	NOS3/AKT1/PPARG/INSR/CASP3/ESR1/MAPK14/BAX/ICAM1/AR/BCL2/PGR/RXRA/HSP90AB1
GO:0001666	response to hypoxia	1.13E-10	13	AKT1/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/KCNMA1/SLC2A4/PSMD3/CC
GO:0097191	extrinsic apoptotic signaling pathway	1.43E-10	11	NOS3/AKT1/HMOX1/GSK3B/RELA/CASP3/BAX/ICAM1/AR/BCL2/GSTP1
GO:0036293	response to decreased oxygen levels	1.64E-10	13	AKT1/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/KCNMA1/SLC2A4/PSMD3/CC
GO:0048511	rhythmic process	1.69E-10	12	NOS3/PPARG/NOS2/GSK3B/CASP3/ESR1/MAPK8/JUN/CDK1/AHR/PGR/HAS2
GO:0071466	cellular response to xenobiotic stimulus	3.18E-10	10	CYP3A4/CYP1A2/ICAM1/GSTM1/GSTP1/AHR/CYP1A1/NR1I2/CCNA2/HSP90AB1
GO:0034612	response to tumor necrosis factor	3.22E-10	12	AKT1/RELA/CASP3/MAPK14/ICAM1/STAT1/IKBKB/VCAM1/GSTP1/SLC2A4/PSMD3/HAS2
GO:0008585	female gonad development	8.55E-10	8	NOS3/INSR/CASP3/ESR1/BAX/ICAM1/BCL2/PGR
GO:0051090	regulation of DNA-binding transcription factor activity	1.09E-09	13	AKT1/PPARG/HMOX1/RELA/PPP3CA/ESR1/MAPK14/MAPK8/JUN/ICAM1/AR/IKBKB/PIM1
GO:0048732	gland development	1.15E-09	13	AKT1/HMOX1/INSR/RELA/ESR1/JUN/XDH/BAX/AR/BCL2/CYP1A1/PGR/RXRA
GO:0046545	development of primary female sexual characteristics	1.29E-09	8	NOS3/INSR/CASP3/ESR1/BAX/ICAM1/BCL2/PGR
GO:2000379	positive regulation of reactive oxygen species metabolic process	1.39E-09	8	AKT1/INSR/MAPK14/XDH/F2/ICAM1/GSTP1/HSP90AB1
GO:2001237	negative regulation of extrinsic apoptotic signaling pathway	1.63E-09	8	NOS3/AKT1/HMOX1/RELA/ICAM1/AR/BCL2/GSTP1

GO:0006979	response to oxidative stress	1.84E-09	13	NOS3/AKT1/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/STAT1/BCL2/GSTP1/CDK2/CCNA2
GO:0071356	cellular response to tumor necrosis factor	2.29E-09	11	AKT1/RELA/MAPK14/ICAM1/STAT1/IKBKB/VCAM1/GSTP1/SLC2A4/PSMD3/HAS2
GO:0051098	regulation of binding	2.45E-09	12	AKT1/PPARG/HMOX1/GSK3B/PPP3CA/MAPK8/JUN/BAX/BCL2/PRKACA/SLPI/HSP90AB1
GO:0018105	peptidyl-serine phosphorylation	3.04E-09	11	AKT1/GSK3B/MAPK14/MAPK8/CDK1/BAX/BCL2/IKBKB/PRKACA/CDK2/HSP90AB1
GO:0048545	response to steroid hormone	3.30E-09	12	PPARG/RELA/CASP3/ESR1/ICAM1/AR/BCL2/NR3C2/GSTP1/PGR/NR1I2/RXRA
GO:0034599	cellular response to oxidative stress	3.37E-09	11	NOS3/AKT1/HMOX1/RELA/MAPK8/JUN/CDK1/BCL2/GSTP1/CDK2/CCNA2
GO:1901653	cellular response to peptide	3.50E-09	12	AKT1/PPARG/INSR/GSK3B/RELA/ICAM1/STAT1/VCAM1/GSTP1/PRKACA/SLC2A4/CCNA2

Table 5 Cellular component of GO

ID	Cellular component	P value	Count	Gene name
GO:0045121	membrane raft	6.06E-13	14	ADRA1B/NOS3/HMOX1/INSR/CASP3/ICAM1/SLC6A2/IKBKB/DPP4/PRKACA/KCNMA1/SLC2
GO:0098857	membrane microdomain	6.33E-13	14	ADRA1B/NOS3/HMOX1/INSR/CASP3/ICAM1/SLC6A2/IKBKB/DPP4/PRKACA/KCNMA1/SLC2
GO:0098589	membrane region	1.05E-12	14	ADRA1B/NOS3/HMOX1/INSR/CASP3/ICAM1/SLC6A2/IKBKB/DPP4/PRKACA/KCNMA1/SLC2
GO:0044853	plasma membrane raft	1.55E-09	8	ADRA1B/NOS3/HMOX1/INSR/PRKACA/KCNMA1/HAS2/SCN5A
GO:0005901	caveola	1.71E-07	6	ADRA1B/NOS3/HMOX1/INSR/KCNMA1/SCN5A
GO:1902911	protein kinase complex	2.19E-05	5	INSR/CDK1/IKBKB/CDK2/CCNA2
GO:0034702	ion channel complex	3.88E-05	7	GABRA1/GABRA2/KCNH2/CALM1/PRKACA/KCNMA1/SCN5A
GO:1902495	transmembrane transporter complex	6.18E-05	7	GABRA1/GABRA2/KCNH2/CALM1/PRKACA/KCNMA1/SCN5A
GO:1990351	transporter complex	7.20E-05	7	GABRA1/GABRA2/KCNH2/CALM1/PRKACA/KCNMA1/SCN5A
GO:0061695	transferase complex, transferring phosphorus-containing groups	0.000147031	6	INSR/CDK1/IKBKB/CDK2/PIK3CG/CCNA2
GO:1902554	serine/threonine protein kinase complex	0.000159203	4	CDK1/IKBKB/CDK2/CCNA2
GO:0032590	dendrite membrane	0.00025657	3	GABRA1/GABRA2/INSR
GO:0005667	transcription regulator complex	0.000285072	7	PPARG/RELA/JUN/AHR/CDK2/NR1I2/RXRA
GO:0000307	cyclin-dependent protein kinase holoenzyme complex	0.000296812	3	CDK1/CDK2/CCNA2
GO:0101002	ficolin-1-rich granule	0.000589532	4	MAPK14/GSTP1/PSMD3/HSP90AB1
GO:1904813	ficolin-1-rich granule lumen	0.000589532	4	MAPK14/GSTP1/PSMD3/HSP90AB1
GO:0034703	cation channel complex	0.000591622	5	KCNH2/CALM1/PRKACA/KCNMA1/SCN5A
GO:0032589	neuron projection membrane	0.000731973	3	GABRA1/GABRA2/INSR
GO:0042383	sarcolemma	0.000833459	4	PPP3CA/VCAM1/SLC2A4/SCN5A
GO:0000790	nuclear chromatin	0.0010602	6	RELA/ESR1/JUN/AR/STAT1/RXRA
GO:1902711	GABA-A receptor complex	0.001556317	2	GABRA1/GABRA2
GO:1902710	GABA receptor complex	0.001725804	2	GABRA1/GABRA2
GO:0090575	RNA polymerase II transcription regulator complex	0.001740876	4	PPARG/JUN/NR1I2/RXRA
GO:0031256	leading edge membrane	0.001898987	4	GABRA1/GABRA2/INSR/DPP4
GO:0046930	pore complex	0.002284373	2	BAX/BCL2

GO:0034774	secretory granule lumen	0.00313051	5	MAPK14/GSTP1/SLPI/PSMD3/HSP90AB1
GO:0032809	neuronal cell body membrane	0.003144133	2	INSR/SLC6A2
GO:0060205	cytoplasmic vesicle lumen	0.003300645	5	MAPK14/GSTP1/SLPI/PSMD3/HSP90AB1
GO:0031253	cell projection membrane	0.003344202	5	GABRA1/GABRA2/INSR/DPP4/HSP90AB1
GO:0044298	cell body membrane	0.00337927	2	INSR/SLC6A2

Table 6 KEGG pathways analysis

ID	Pathway	P value	Count	Gene name
hsa05418	Fluid shear stress and atherosclerosis	1.43E-14	15	NOS3/AKT1/HMOX1/RELA/CALM1/MAPK14/MAPK8/JUN/ICAM1/GSTM1/BCL2/IKBKB/VCAM1/GS
hsa04933	AGE-RAGE signaling pathway in diabetic complications	8.81E-14	13	NOS3/AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/BCL2/VCAM1/PIM1
hsa05167	Kaposi sarcoma-associated herpesvirus infection	2.98E-11	14	AKT1/GSK3B/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/IKBKB/PIK:
hsa05169	Epstein-Barr virus infection	5.51E-11	14	AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/BCL2/IKBKB/CDK2/PSMD3/CCNA2
hsa05161	Hepatitis B	7.35E-10	12	AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/STAT1/BCL2/IKBKB/CDK2/CCNA2
hsa05170	Human immunodeficiency virus 1 infection	1.36E-09	13	AKT1/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/JUN/CDK1/BAX/BCL2/IKBKB/CHEK1
hsa05162	Measles	2.03E-09	11	AKT1/GSK3B/RELA/CASP3/MAPK8/JUN/BAX/STAT1/BCL2/IKBKB/CDK2
hsa04659	Th17 cell differentiation	2.33E-09	10	RELA/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB/AHR/RXRA/HSP90AB1
hsa05145	Toxoplasmosis	3.66E-09	10	AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/STAT1/BCL2/IKBKB/PIK3CG
hsa04722	Neurotrophin signaling pathway	6.63E-09	10	AKT1/GSK3B/RELA/CALM1/MAPK14/MAPK8/JUN/BAX/BCL2/IKBKB
hsa05222	Small cell lung cancer	1.06E-08	9	AKT1/NOS2/RELA/CASP3/BAX/BCL2/IKBKB/CDK2/RXRA
hsa04657	IL-17 signaling pathway	1.28E-08	9	GSK3B/RELA/CASP3/MAPK14/MAPK8/JUN/MMP1/IKBKB/HSP90AB1
hsa05215	Prostate cancer	1.70E-08	9	AKT1/GSK3B/RELA/AR/BCL2/IKBKB/GSTP1/CDK2/HSP90AB1
hsa04914	Progesterone-mediated oocyte maturation	2.22E-08	9	AKT1/MAPK14/MAPK8/CDK1/PRKACA/CDK2/PGR/CCNA2/HSP90AB1
hsa05166	Human T-cell leukemia virus 1 infection	2.28E-08	12	AKT1/RELA/PPP3CA/MAPK8/JUN/BAX/ICAM1/IKBKB/PRKACA/CDK2/CHEK1/CCNA2
hsa05152	Tuberculosis	3.09E-08	11	AKT1/NOS2/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/BAX/STAT1/BCL2
hsa04625	C-type lectin receptor signaling pathway	3.15E-08	9	AKT1/RELA/CALM1/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB
hsa04931	Insulin resistance	4.39E-08	9	NOS3/AKT1/INSR/GSK3B/RELA/MAPK8/IKBKB/SLC2A4/PYGM
hsa04668	TNF signaling pathway	6.03E-08	9	AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/IKBKB/VCAM1
hsa04932	Non-alcoholic fatty liver disease	6.20E-08	10	AKT1/INSR/GSK3B/RELA/CASP3/MAPK8/JUN/BAX/IKBKB/RXRA
hsa04380	Osteoclast differentiation	1.92E-07	9	AKT1/PPARG/RELA/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB
hsa04926	Relaxin signaling pathway	2.06E-07	9	NOS3/AKT1/NOS2/RELA/MAPK14/MAPK8/JUN/MMP1/PRKACA
hsa01522	Endocrine resistance	3.10E-07	8	AKT1/ESR1/MAPK14/MAPK8/JUN/BAX/BCL2/PRKACA
hsa04910	Insulin signaling pathway	3.45E-07	9	AKT1/INSR/GSK3B/CALM1/MAPK8/IKBKB/PRKACA/SLC2A4/PYGM
hsa04915	Estrogen signaling pathway	3.67E-07	9	NOS3/AKT1/CALM1/ESR1/JUN/BCL2/PRKACA/PGR/HSP90AB1
hsa04917	Prolactin	4.48E-	7	AKT1/GSK3B/RELA/ESR1/MAPK14/MAPK8/STAT1

	signaling pathway	07		
hsa04660	T cell receptor signaling pathway	4.91E-07	8	AKT1/GSK3B/RELA/PPP3CA/MAPK14/MAPK8/JUN/IKBKB
hsa01524	Platinum drug resistance	5.99E-07	7	AKT1/CASP3/BAX/GSTM1/BCL2/GSTP1/TOP2B
hsa04261	Adrenergic signaling in cardiomyocytes	7.45E-07	9	ADRA1B/ADRA1D/AKT1/CALM1/MAPK14/BCL2/PRKACA/PIK3CG/SCN5A
hsa05133	Pertussis	7.91E-07	7	NOS2/RELA/CASP3/CALM1/MAPK14/MAPK8/JUN

Table 7 Virtual Docking Of Four Bioactive Ingredients From Acoritataninowii Rhizoma For AD Targets

Ingredients	Structure	Binding Energy/(kcal•mol ⁻¹)				
		AKT1	CASP3	ESR1	JUN	MAPK14
kaempferol		-6.48	-6.14	-6.92	-6.45	-6.63
8-Isopentenyl-kaempferol		-6.04	-6.96	-5.65	-6.31	-7.1
(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan		-6.4	-7.9	-7.22	-7.33	-6.81
Cycloartenol		-9.07	-7.57	-10.85	-8.98	-9.29

Figures

Figure 1

The schedule in the present study was outlined as followed: (1) the ingredients of *Acoritataninowii Rhizoma* along with their corresponding targets and AD associated targets were searched and screened from databases; (2) The network of medicine-ingredients-targets-disease was constructed, visualized, and analyzed; (3) key ingredients, core targets, top functions and signal pathways were analyzed by PPI, GO, KEGG and the networks were constructed; (4) the valuable targets were obtained and validated via molecular docking with corresponding ingredients and the top targets.

Figure 2

The network of medicine-ingredients-targets. The gene targets were described as green oval. yellow octagon represents ingredients. pastel pink diamond was employed to stand for Acoritataninowii Rhizoma. The edges stand for the association between the nodes.

Figure 3

The potential target genes in Acoritaninowii Rhizoma and AD were shown as purple circle and yellow circle in the intersection, respectively (A). In addition, the network of medicine-ingredients-targets-disease was shown as followed: the gene targets (green oval), ingredients (pastel pink rectangle), Acoritaninowii Rhizoma (yellow diamond) and AD (blue hexagon) (B). The edges stand for the association between the nodes.

Figure 4

The interaction of overlapping target proteins between Acoritaninowii Rhizoma and AD were shown by network of PPI (B) and the node degree of proteins were analyzed (A).

A

B

Figure 5

The molecular function from GO enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, GO enrichment in the top 30 from the molecular function had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (green oval) and molecular functions (pink diamond) (B).

A

B

Figure 6

The biological process from GO enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, GO enrichment in the top 30 from the molecular function had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (green hexagon) and molecular functions (yellow rectangle) (B).

Figure 7

The cellular components from GO enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, GO enrichment in the top 30 from the molecular function had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (blue rectangle) and molecular functions (green oval) (B).

Figure 8

KEGG enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, signaling pathways from KEGG enrichment had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (purple rectangle) and signaling pathways (pink oval) (B).

Figure 10

Kaposi sarcoma-associated herpesvirus infection