

# Network Pharmacology-Based Analysis on the Mechanism of Drug Pair of Astragalus Membranaceus and Acorus Tatarinowii in the Treatment of Alzheimer's Disease

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**Abstract.** Alzheimer's disease (AD) is a degenerative disease of central nervous system, which seriously threatens the life and health of the elderly people. It has been for long time that Traditional Chinese medicine (TCM) treatment for AD is effective. This study analyzed the potential target and molecular mechanism of the most often used drug pair of Astragalus membranaceus and Acorus tatarinowii to treat AD by network pharmacological method. Firstly, the method was performed to screen and sort out the active ingredients with good ADME properties and drug targets of Astragalus membranaceus and Acorus tatarinowii. Then, we searched for the disease targets related to AD, followed by the construction of the "active ingredients-target-disease" network. We implemented GO enrichment analysis and KEGG pathway enrichment analysis of related overlapped target proteins, and then constructed the "target-pathway" network diagram. Finally, the above overlapped target proteins are mapped to build a PPI high-position protein interoperability network, and we conducted the network topology analysis to screen out the core targets of Astragalus membranaceus-Acorus tatarinowii drug pair in the treatment of AD. According to network pharmacology, this research predicted the potential targets of the drug pair of Astragalus membranaceus and Acorus tatarinowii in the treatment of AD, and explored that Astragalus membranaceus-Acorus tatarinowii drug pair in the treatment of AD was the overall systematic regulating action of "multiple-ingredients, multiple-target and multiple-pathway". It affords the reference for understanding the pathogenesis of AD and exploring new therapeutic methods and drug development in the future.

**Keywords.** Alzheimer's disease, the network pharmacology, Astragalus membranaceus, Acorus tatarinowii, mechanism

## 1. Introduction

Alzheimer's disease (AD) is the main types senile dementia, accounting for about 60% of senile dementia [1]. Its clinical manifestations including progressive memory decline, cognitive dysfunction, other neurological problems and behavioral disorders [2].

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Studies have found that age is the main factor in the onset of AD in patients with AD. Among people aged 50-80 years, the prevalence of AD will double for every 5-year increase in age [3]. According to the epidemiological survey, with the rising trend of population aging in China, the incidence of AD will increase significantly. It is expected that by 2030, there will be more than 10x10<sup>6</sup> cases of AD in China [4].

The pathological features of AD are Tau protein deposition [5] and intracellular neurofibrillary tangles [6]. At present, there are several theories about the pathogenesis of AD: cholinergic deficiency [7], hyperphosphorylation of Tau [8], oxidative stress and excessive deposition of A $\beta$  [9]. These theories provide an overview of the pathogenesis of AD from different aspects, but the etiology of the disease is extremely complex and its pathogenesis remains unclear.

The modern medical treatment of AD is mainly based on drug, supplemented by cognitive training. Medicine therapy mainly includes improving brain microcirculation, regulating nerve cell metabolism, reducing oxidative stress, repairing nerve fibers and so on. Common drugs for the treatment of AD include Donepezil [10], Sodium oligomannate [11], VitaminC [12] and Memantine [13]. Although modern western medicine can improve some symptoms of AD patients, it cannot fundamentally treat AD [14]. At present, some studies showed that Chinese herbal medicine can effectively treat AD, such as Ginseng therapy, which protects neurons and improves cognitive function [15]. *Salvia miltiorrhiza* Bunge reduces Tau hyperphosphorylation and prevents the formation and decomposition of A $\beta$  fiber [16].

*Astragalus membranaceus* has the efficacy of improving body immunity, anti-aging and anti-oxidation in the modern pharmacological study. Studies have found that *Astragalus polysaccharide* can alleviate the process of AD by downregulating the expression levels of APP, A $\beta$ , PTAu, GSK3 $\beta$  and BACE1 in the hippocampus of AD model rats and up-regulating the protein expression level of PP2A, and can also significantly improve the positioning and navigation ability and learning and memory ability of rats [17]. The extracts, volatile oil and asarone ether of *Acorus tatarinosus* have sedative, anti-convulsant, anti-depression, improving learning and memory and anti-brain damage effects. Tian et al. [18] studied the mechanism of different effective parts of *Acorus tatarinowii*, improving the cognitive function of AD model mice, and found that the cognitive function of model mice was improved by gavage of different effective parts of *Acorus tatarinowii*, and the water extract and volatile oil gavage group improved the cognitive function of mice most significantly. Song [19] et al. conducted data mining on TCM prescriptions for the treatment of AD in the past 30 years. According to the research, among the 241 first prescriptions based on the statistics of "ancient and modern medical case cloud platform V1.4.1", the top five are *Acorus tatarinowii*, *Astragalus membranaceus*, *Rehmannia glutinosa*, *Salvia miltiorrhiza*, and *Poria*; The most common drug pairs are "Acorus tatarinowii-Polygala", "Acorus tatarinowii-Astragalus membranaceus" and "Acorus tatarinowii-Salvia miltiorrhiza". Based on the analysis of the law of TCM for 241 prescriptions, the confidence and correlation of the three-drug pairs are still in the top three.

Network pharmacology analyzes the multiple-level and multiple-angle biological network relationship among "medicine-gene-target-disease" and the relationship between different ingredients of TCM. It also studies the biological pathway signals of diseases, and establishes the overall relationship between active ingredients and targets and diseases. The integrity, systematization and emphasis on the interaction between drugs of the network pharmacological analysis are consistent with the characteristics of

TCM, so as it can confirm potential drug targets and clarify the mechanism of drug action [20].

This study purposed to analyze the potential targets and molecular mechanisms of action of Astragalus membranaceus-Acorus tatarinowii drug pair in the treatment of AD by the network pharmacological method. It supplies a theoretical foundation and research strategies for future research on the pathogenesis of AD, as well as the research on new therapeutic methods and drug development.

## 2. Materials and Methods

### 2.1. Screening of Active Ingredients of Drug Pair

This paper used the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) (<http://tcmssp.com/tcmssp.php>) to collect the active ingredients of Astragalus membranaceus and Acorus tatarinowii. Taking “Astragalus membranaceus” and “Acorus tatarinowii” as the search key words, Drug-likeness ( $DL \geq 0.18$ ), Blood-brain barrier ( $BBB \geq 0.3$ ), and Oral Bioavailability ( $OB \geq 30\%$ ) as the search conditions, then acquired the active ingredients of Astragalus membranaceus and Acorus tatarinowii for treating AD.

### 2.2. Collection of Disease Targets Related to Alzheimer's Disease

To explore the relevant disease targets of AD, we used the DisGeNET (<http://www.Disgenet.org/>) database to obtain the target genes related to AD by using the medical keyword “Alzheimer's Disease”. Subsequently, sort out the collected relevant disease targets, and then obtain the relevant known human targets of AD and save them in TXT format.

### 2.3. Prediction and Screening of Drug Targets

SwissTargetPrediction database (<http://www.swisstargetprediction.ch/>) was selected to find the top 30 active components of Astragalus membranaceus and Acorus tatarinowii. In addition, the TCMSP database and SwissTargetPrediction database were integrated to collect the targets of active ingredients of Astragalus membranaceus and Acorus tatarinowii. After redundancy was removed, all the collected targets were imported into bioDBnet software (<https://biodbnet-abcc.ncifcrf.gov/>) for batch standardization, and screen for non-human targets and calibrate related target names. Then, we obtained the target data set of drug active ingredients and saved it in TXT format.

### 2.4. Construction and Analysis of Active Ingredient-Target-Disease Network Diagram

To elucidate the molecular mechanism of drug pair to treat AD, we constructed a visual network of “ingredients-target-disease” interaction based on the collection of drug targets and disease targets. The collected and sorted Astragalus membranaceus and Acorus tatarinowii ingredients targets were mapped against each other with AD targets to screen out the common composition-disease targets. The screened targets were uploaded to Cytoscape 3.7.2 software (<http://www.cytoscape.org/>), and then the

visualization function was used to construct the “Astragalus membranaceus and Acorus tatarinowii “active ingredients-target- disease” network map and save it.

### 2.5. GO and KEGG Analysis and the Construction of Target-Pathway Network Diagram

In this study, we analyzed GO enrichment analysis and KEGG pathway enrichment analysis of drug pair and disease target of AD by using the clusterProfiler enrichment function in R language, and drew bubble charts and bar charts. On color represented on type of enrichment results, their sizes were positively correlated with the significance. Then we used Cytoscape 3.7.2 visualization software to visualize the signal pathways and the target to construct a “target-pathway” network.

### 2.6. Construction of Protein Interaction Network and Network Topology Analysis

The protein-protein interaction (PPI) network has become an important method to find direct and indirect interactions between targets and targets. Therefore, this study aimed to screen the core targets of Astragalus membranaceus and Acorus tatarinowii in the treatment of AD, using Cytoscape 3.7.2 software. The potential target information was imported into Cytoscape 3.7.2 software, and the high-confidence PPI network information of the active ingredients of Astragalus membranaceus and Acorus tatarinowii and AD intersection targets were obtained based on the BioGRID function package. Meanwhile, based on this high-confidence PPI network, Network topology Analysis was performed using the CytoNCA toolkit network analysis to filter core targets based on relevant topology parameters.

## 3. Results

### 3.1. Screening of Active Ingredients of Drug Pair

In this study, 14 active ingredients of Astragalus membranaceus and 2 active ingredients of Acorus calamus were acquired through TCMSP database and literature search. Detailly, the chemical information of each ingredient was tabled according to the number, name, OB value, BBB value, and DL value, and the results were used to construct the dataset of active ingredient information of Astragalus membranaceus and Acorus tatarinowii. Subsequently, we screened the highly active pharmaceutical ingredients according to the criteria of  $OB \geq 30\%$ ,  $BBB \geq 0.3$  and  $DL \geq 0.18$ , and obtained 14 active ingredients of Astragalus membranaceus and 2 active ingredients of Acorus tatarinowii, add up to 6 nonrepetitive effectively active ingredients with good ADME properties (table 1).

### 3.2. Identification of Alzheimer's Disease Targets

All target genes associate with AD were searched and converged by the GeneCards and DisGeNET database with “Alzheimer's Disease” as the keyword. We processed and integrated the collected disease targets, deleted redundant and duplicate targets, collected 3397 AD targets, sorted and tabulated them and saved them in TXT format.

**Table 1.** Basic information of Astragalus membranaceus and Acorus tatarinowii effectively active ingredients.

Molecular number	Effectively active ingredients name	OB	BBB	DL
MOL000211	Mairin	55.38	0.22	0.78
MOL000239	Jaranol	50.83	-0.22	0.29
MOL000296	Hederagenin	36.91	0.96	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	1.09	0.78
MOL000371	3,9-di-O-methylinissolin	53.74	0.63	0.48
MOL000378	7-O-methylisomucronulatol	74.69	0.84	0.3
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.55	0.42
MOL000387	Bifendate	31.1	-0.06	0.67
MOL000392	Formononetin	69.67	0.02	0.21
MOL000398	Isoflavanone	109.99	0.17	0.3
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.34	0.26
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	-0.04	0.48
MOL003576	(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-Hexahydrofuro[4,3-c]furan	52.35	0.83	0.62
MOL003578	Cycloartenol	38.69	1.53	0.78
MOL003576	(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-Hexahydrofuro[4,3-c]furan	52.35	0.83	0.62
MOL003578	Cycloartenol	38.69	1.53	0.78

### 3.3. Screening and Identification of Drug Targets

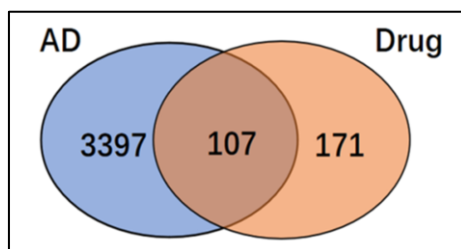
A total of 229 Astragalus membranaceus and 69 Acorus tatarinowii drug targets were found through the use of TCMSP and SwissTargetPrediction database. After standardization of bioDBnet software and dedundancy, finally, a total of 171 Astragalus membranaceus and Acorus tatarinowii drug targets were collected by standardization of bioDBnet software and dedundancy.

### 3.4. Construction of “Active Ingredients-Target-Disease” Network Map and Analysis of Results

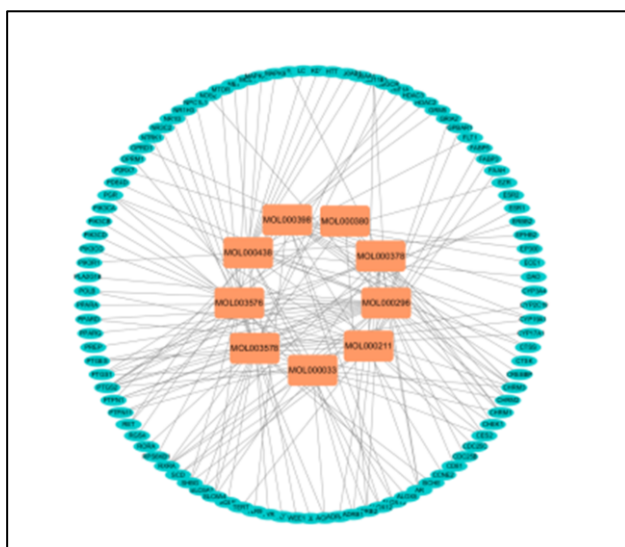
The Astragalus membranaceus and Acorus tatarinowii drug targets were mapped against the collected AD targets to acquire the targets of the drugs active ingredients of AD treatment. The drug and disease targets were uploaded to Venny to draw a Venn diagram of the common action targets of drugs and diseases. Finally, we acquired 107 common active targets (figure 1).

The “active ingredient-target-Alzheimer’s disease” network was established by Cytoscape3.7.2. Figure 2 represents the 16 active ingredients in Astragalus membranaceus and Acorus tatarinowii that interact with 107 potential targets and thus affect the development of AD, of which only 9 active ingredients can interact with potential targets, they are Mairin;Hederagenin; Isoflavanone;Cycloartenol;(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol;(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-Hexahydrofuro[4,3-c]furan;(6aR,11aR)-9,10-

dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol;7-O-methylisomucronulatol;(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol. The results showed that Astragalus membranaceus-Acorus tatarinowii drug pair play an effect on the cure of AD by multiple targets, multiple chemical ingredients, and comprehensive regulation.



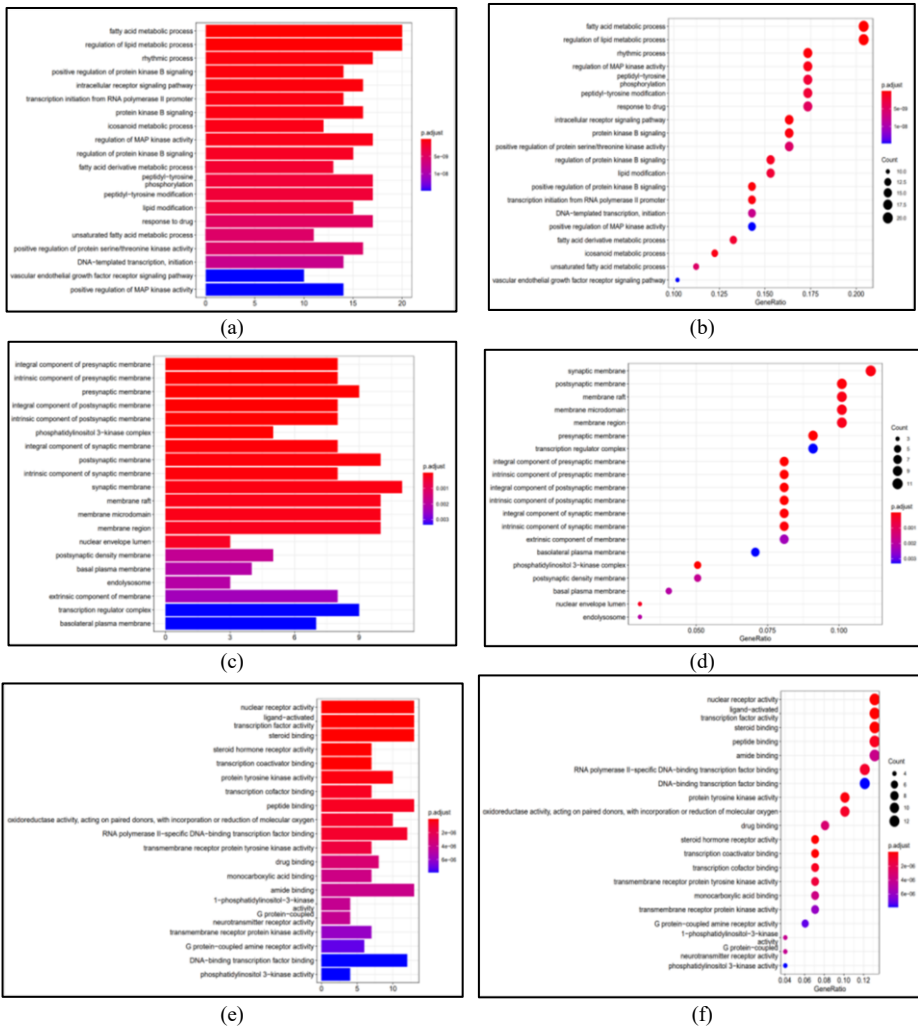
**Figure 1.** The Venn diagram of the common target of drugs and diseases. Blue is the disease target of Alzheimer's disease; orange is the drug target of Astragalus membranaceus and Acorus tatarinowii; the intersecting part is the common target.



**Figure 2.** Ingredients-target-disease interaction network diagram. Orange rectangles part is the relevant active ingredient; green ellipse part is the relevant target.

### 3.5. GO Function Enrichment Analysis Results

We used the GO enrichment analysis function in R language to research the 107 target protein-related genes functions involved in the above-mentioned “ingredients-target-disease” network diagram, then the 60 GO items were selected in the GO enrichment analysis and drawn corresponding bar charts and bubble charts. The results are shown in figure 3 and table 2. As can be seen from table 2, the table involves three parts: biological process (BP), cell composition (CC) and molecular function (MF), and each part selects 10 main go items.



**Figure 3.** Histograms and bubble charts of GO enrichment analysis of potential targets of *Astragalus membranaceus* and *Acorus tatarinowii* drug pair related to AD. Note, (a) and (b) are bar and bubble chart of BP respectively. (c) and (d) are CC bar and bubble chart respectively. (e) and (f) are MF bar and bubble chart respectively.

### 3.6. KEGG Functional Enrichment Analysis Results and the Construction of Target-Pathway Network Diagram

To illustrate the role of active components of *Astragalus membranaceus*-*Acorus tatarinowii* drug pair to treat of AD-related targets in the signaling pathway, the 107 potential targets in the above network diagram through the KEGG enrichment analysis function in R language. The 20 KEGG items were selected in the KEGG enrichment analysis and drawn corresponding bar charts and bubble charts. The 20 signaling pathways obtained are involved in prostate cancer, central carbon metabolism of cancer, thyroid hormone signaling pathway, cAMP signaling pathway, and so on (figure 4). These results indicate that the active ingredients of *Astragalus membranaceus* and *Acorus tatarinowii* can affect the development of AD disease through signal pathways such as the thyroid signaling pathway. Further, 107 potential target genes and the involved KEGG pathways were introduced into the software of Cytoscape3.7.2 to construct a “target-pathway” network map, as shown in figure 5.

**Table 2.** Major GO entries of potential targets of Astragalus membranaceus and Acorus tatarinowii drug pair related to AD.

Category	Term identification number	Function	Pvalue	Qvalue	Count
GOTERM_BP_DIRECT	GO:0006631	Fatty acid metabolism process	1.31E-14	2.41E-11	20
GOTERM_BP_DIRECT	GO:0019216	Regulation of lipid metabolism process	6.44E-14	5.94E-11	20
GOTERM_BP_DIRECT	GO:0048511	Rhythmic process	3.17E-13	1.82E-10	17
GOTERM_BP_DIRECT	GO:0051897	Positive regulation of protein kinase B signaling	4.33E-13	1.82E-10	14
GOTERM_BP_DIRECT	GO:0030522	Intracellular receptor signaling pathway	4.95E-13	1.82E-10	16
GOTERM_BP_DIRECT	GO:0006367	Transcription initiation of RNA polymerase II promoter	9.88E-13	2.71E-10	14
GOTERM_BP_DIRECT	GO:0043491	Protein kinase B signaling	1.03E-12	2.71E-10	16
GOTERM_BP_DIRECT	GO:0006690	Ionic metabolic process	1.57E-12	3.61E-10	12
GOTERM_BP_DIRECT	GO:0043405	Regulation of MAP kinase activity	2.00E-12	4.10E-10	17
GOTERM_BP_DIRECT	GO:0051896	Regulation of protein kinase B signal transduction	3.65E-12	6.73E-10	15
GOTERM_CC_DIRECT	GO:0099056	The composition of the presynaptic membrane	3.70E-09	6.82E-07	8
GOTERM_CC_DIRECT	GO:0098889	Intrinsic ingredients of presynaptic membrane	9.33E-09	8.59E-07	8
GOTERM_CC_DIRECT	GO:0042734	Presynaptic membrane	9.73E-08	5.97E-06	9
GOTERM_CC_DIRECT	GO:0099055	The composition of the postsynaptic membrane	1.40E-07	6.45E-06	8
GOTERM_CC_DIRECT	GO:0098936	Intrinsic ingredients of the postsynaptic membrane	1.94E-07	7.14E-06	8
GOTERM_CC_DIRECT	GO:0005942	Phosphatidylinositol 3-kinase complex	3.24E-07	9.93E-06	5
GOTERM_CC_DIRECT	GO:0099699	Ingredients of the synaptic membrane	1.15E-06	3.03E-05	8
GOTERM_CC_DIRECT	GO:0045211	Postsynaptic membrane	1.56E-06	3.60E-05	10
GOTERM_CC_DIRECT	GO:0099240	Synaptic membrane ingredients	2.02E-06	4.14E-05	8
GOTERM_CC_DIRECT	GO:0097060	Synaptic membrane	4.19E-06	7.72E-05	11
GOTERM_MF_DIRECT	GO:0004879	Nuclear receptor activity	7.74E-19	1.02E-16	13
GOTERM_MF_DIRECT	GO:0098531	Ligand activates transcription factor activity	7.74E-19	1.02E-16	13
GOTERM_MF_DIRECT	GO:0005496	Steroid binding	1.53E-14	1.34E-12	13
GOTERM_MF_DIRECT	GO:0003707	Steroid Hormone Receptor	6.48E-11	4.27E-09	7
GOTERM_MF_DIRECT	GO:0001223	Transcriptional coactivator binding	1.52E-10	7.99E-09	7
GOTERM_MF_DIRECT	GO:0004713	Protein tyrosine kinase activity	2.96E-09	1.30E-07	10
GOTERM_MF_DIRECT	GO:0001221	Transcription cofactor binding	1.02E-08	3.64E-07	7
GOTERM_MF_DIRECT	GO:0042277	Peptide binding	1.11E-08	3.64E-07	13
GOTERM_MF_DIRECT	GO:0016705	Oxidoreductase activity, acting on the incorporation or reduction of molecular oxygen in pairs of donors	1.72E-08	5.04E-07	10
GOTERM_MF_DIRECT	GO:0061629	RNA polymerase II specific DNA binding transcription factor binding	2.11E-08	5.56E-07	12



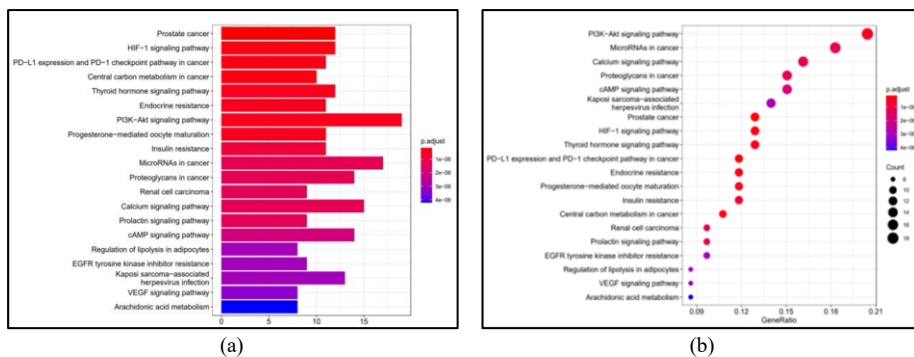


Figure 4. bar chart and bubble chart of KEGG enrichment analysis of drug pair.

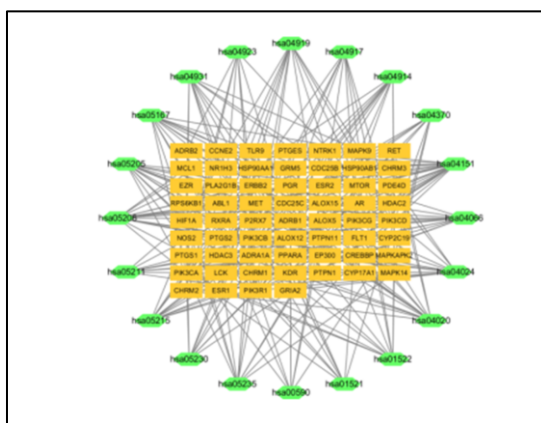
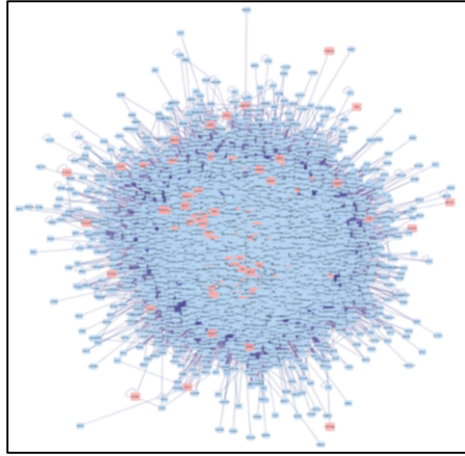


Figure 5. “Target-pathway” network diagram. Yellow rectangles part is the target enriched to the signal pathway; green ellipse part is the signal pathway.

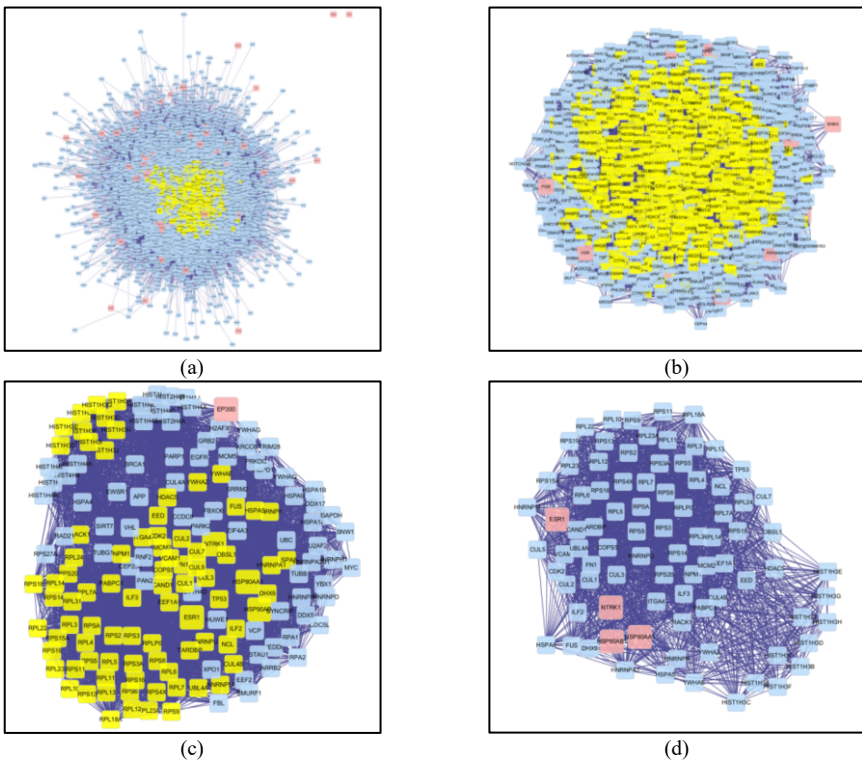
### 3.7. Results of Protein Interaction Network and Network Topology Analysis

In order to screen the core targets of *Astragalus membranaceus* and *Acorus tatarinowii* for the treatment of AD, 107 potential targets were imported into Cytoscape 3.7.2 software (<http://www.cytoscape.org/>). Next, high confidence PPI network information of active ingredients *Astragalus membranaceus* and *Acorus tatarinowii* and disease intersection targets were obtained by Bisogenet function package (figure 6). Based on this PPI network in Cytoscape, the network was topological analyzed though the CytoNCA toolkit, and the corresponding topological parameters DC, BC, NC were 61, 458, 34. The core targets were screened based on the degree centrality (DC) value before betweenness centrality (BC) and drawn a network diagram, and the network graph was drawn (figure 7). Figure 7 is the network topology analysis diagram. The results showed that the red nodes ESR1 (estrogen receptor 1), HSP90AB1 (heat shock protein 90 alpha family class B member 1), NTRK1 (neurotrophic tyrosine kinase receptor 1) and HSP90AA1 (heat shock protein 90 alpha family class A member 1), and are core target proteins, and the yellow nodes are related genes of the core target proteins. Moreover, topological parameters of HSP90AA1, HSP90AB1, NTRK1 and

ESR1 meet the above card values, so they can be used as the core targets of *Astragalus membranaceus* and *Acorus tatarinowii* in the treatment of AD.



**Figure 6.** High-confidence interaction network diagram (PPI) of target proteins of *Astragalus membranaceus* and *Acorus tatarinowii* in the treatment of AD.



**Figure 7.** Core target map of *Astragalus membranaceus* and *Acorus tatarinowii* in the treatment of AD in topological analysis. Note, Red rectangles part is the input target gene; yellow rectangles part is the input target related protein gene A; Based on PPI network and according to the screening criteria of  $DC > 61$ ; B: (a) creates a subnetwork and draws it according to the screening criteria of  $BC > 458$ ; C: (b) creates a subnetwork and draw it according to the standard of  $NC > 34$ ; D: (c) creates a subnetwork.

#### 4. Discussion

AD is a neurodegenerative disease with progressive cognitive decline, which has become the third major killer to human life and health after tumor and cardiovascular disease [21, 22]. AD seriously affects the mental and physical health of the elderly. Therefore, it is important that studying the pathogenesis of AD to its prevention and treatment. In modern medicine, western medicine therapy can only delay and improve the occurrence of symptoms, but can't completely cure the disease. As an auxiliary therapy to treat AD, TCM has the advantage of anti-AD additives, and the well-tolerated ingredients are safer in AD patients. None of these properties exist in currently approved drugs [23]. Therefore, it is of great value to analyze the medication law of TCM therapy AD.

In this study, the TCMSp and SwissTargetPrediction database were used to screen all the effective active components and related targets related to *Astragalus membranaceus* and *Acorus tatarinowii* drug pair, and then the targets were standardized by bioDBnet and other softwares. We collected the target genes of AD through DisGeNET database, and mapped to the drug and disease targets, and drawn the "ingredients-target-disease" network diagram, and constructed the PPI protein interaction network, CytoNCA network topology analysis, GO function enrichment analysis, KEGG signal pathway enrichment analysis, and KEGG relationship network diagram.

The "active ingredients-targets-disease" network diagram (figure 2) showed that *Astragalus membranaceus* and *Acorus tatarinowii* could treat AD by acting on 107 targets, including NTRK1, ESR1, AKR1B10, PTPN1, HSD11B1, PTGES, NR1H4 and so on. The screened potential drug targets may be one of the causes of AD. To further explain the role of gene functions and signaling pathways involved in the related targets of *Astragalus membranaceus* and *Acorus tatarinowii* in the treatment of AD, the potential targets of drug pair to treat of AD were analyzed by GO analysis and KEGG analysis. GO analysis results (figure 3) and KEGG analysis results (figure 4) revealed that relevant drug targets mainly concentrated in the central carbon metabolism, vascular endothelial growth factor and fatty acid metabolism signaling pathways of cancer. And a large number of experiments have revealed that the emerge of AD is strongly linked to cancer diseases and vascular factors. The above results showed that *Astragalus membranaceus* and *Acorus tatarinowii* treated AD comprehensively through multiple ways and multiple pathways. The results also conform to the features of "multi-ingredients and multi-target and multi-pathway" in TCM therapy, and afford a theoretical reference for the subsequent study of AD.

In this study, in order to clarify the core targets of *Astragalus membranaceus*-*Acorus tatarinowii* drug pair in the treatment of AD, we built a high confidence PPI network of *Astragalus membranaceus* and *Acorus tatarinowii* to treat AD by the method of network pharmacology (figure 6), and analyzed the network topology (figure7). The results showed that ESR1, NTRK1, HSP90AA1 and HSP90AB1 were the core targets of *Astragalus membranaceus* and *Acorus tatarinowii* in the treatment of AD. These targets are possible etiologic factors in the pathogenesis of AD.

In addition, this study still has certain limitations. For example, when we used bioinformatics tools to retrieve the root of *Astragalus membranaceus* and *Acorus tatarinowii* and AD targets, the introduction of a large amount of data still forecast target related active ingredients. Therefore, the follow-up research *Astragalus membranaceus* and *Acorus tatarinowii* to treat AD the key targets of therapy and

related pathways also only to predict, and it is still short of follow-up molecular dynamics study and animal experimental support. Based on the method of network pharmacology, this study explores how traditional Chinese medicine can treat *Astragalus membranaceus* and *Acorus tatarinowii* with the features of “multi-ingredients and multi-targets and multi-pathway” to treat AD systematically. This experiment provides the theoretical basis and research ideas for studying the pathogenesis and drug development of AD.

## 5. Conclusion

In this research, we used bioinformatics tools to search the targets of *Astragalus membranaceus*, *Acorus tatarinowii* and AD, and classified and sorted them, and finally predicted the key targets and related pathways of *Astragalus membranaceus* and *tatarinowii* in the treatment of AD.

## Acknowledgments

The study was supported by Liaoning provincial key R & D project (No. 2020JH2/10300114 and 2019JH8/10300057).

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