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Original Article

Potential Targets and Pharmacological Effects of Wuling Capsule on Alzheimer's Disease: A Network Pharmacology-based Analysis

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Abstract

Background and objectives: Alzheimer's disease (AD) is a common geriatric disease with a complex pathogenesis and challenging treatment options. Wuling capsule is a single herbal formula mainly composed of *Xylaria nigripes* powder, which has sedative and neuroprotective effects on the central nervous system. This study aimed to explore various potential pathways and targets of Wuling capsules for the treatment of AD.

Methods: The anti-AD mechanism of Wuling capsule was systematically analyzed by integrating multiple databases and using network pharmacology. The active ingredients of Wuling capsules were screened through the Pubchem website, the SwissADME database, and a literature search. The related targets of AD were then screened in the GeneCards database. Using Cytoscape software and STRING, the disease-drug-target interaction network and the protein-protein interaction network were visualized, and topological analysis revealed the differences in the effects of different types of compounds.

Results: Fifty-four compounds and 284 targets were screened by network pharmacology. The main active ingredients included quercetin, xylaric acid A-D, lysine, gamma-aminobutyric acid, glutamic acid, other amino acids, trace elements, guanosine, adenosine, etc. The targets in the network cover inflammation, oxidative stress, modulation of chemical synaptic transmission, and other related proteins, including protein kinase B, tumor necrosis factor-alpha, and tumor suppressor p53. The enrichment analysis results showed that these pathways include the phosphoinositide-3-kinase/protein kinase B, mitogen-activated protein kinase, and tumor necrosis factor-alpha signaling pathways. We also explored five potential protein functional modules.

Conclusions: This study revealed the multi-target and multi-pathway effects of the drug-ingredient-target-disease network through network pharmacology. This systematic screening strategy provides a new concept and theoretical basis for the treatment of AD with Wuling capsules.

Keywords: Wuling capsule; Alzheimer's disease; Network pharmacology; Neuroprotection; Inflammation.

Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer's disease; AKT, protein kinase B; GO, Gene Ontology; IL, interleukin; KEGG, Kyoko Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3-kinase; PPI, protein-protein interaction; TNF, tumor necrosis factor; WLJ, Xylaria nigripes.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized clinically by memory impairment, aphasia, apraxia, cognitive impairment, visual-spatial skill impairment, executive dysfunction, and behavioral changes.[1] Pathological mechanisms include the accumulation of the β-amyloid peptide (Aβ), hyperphosphorylated tau, synaptic plasticity, oxidative stress, inflammation, and glucose metabolism. In recent years, with the increasing aging of the population, the prevalence and mortality of AD have been increasing year by year.[2] According to reports, an estimated 6.2 million Americans aged 65 and older are currently living with AD, and by 2050, the number of people with dementia will triple worldwide.[3] The increase in the number of patients is accompanied by an increase in nursing costs and social

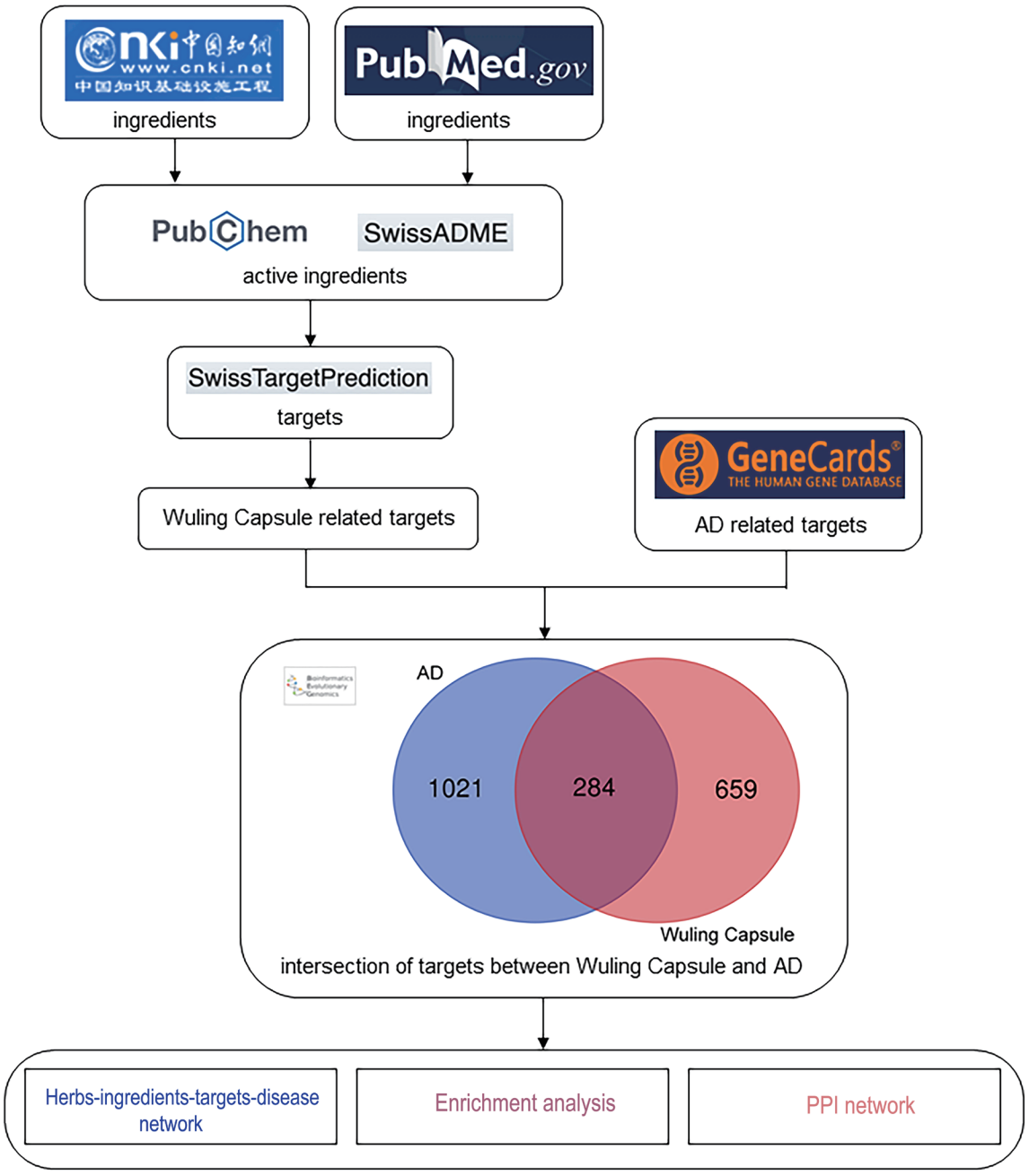

Fig. 1. A description of the workflow for studying Wuling capsules for the treatment of AD. AD, Alzheimer's disease.

medical pressures.

Wuling capsules are mainly composed of Xylaria nigripes powder, also known as Wu Ling Shen, which is a very valuable medicinal fungus. Studies have shown that Xylaria nigripes contains adenosine, adenine, uridine, guanosine, polysaccharide, mannitol, ergosterol, cholesterol, β-sitosterol and other nucleosides, polysaccharides, sterols, and other components, which have anti-oxidant, anti-inflammatory and antitumor activity.[4,5] In recent years, many studies have shown that Xylaria nigripes can act on the nervous system to treat diseases such as epilepsy, depression, post-traumatic stress disorder, and cognitive impairment.[5–7] However, the current research on Wuling capsules for AD lacks a systematic and comprehensive explanation. Therefore, in this study, we used network pharmacology to illustrate the interaction among drugs, targets, and diseases. Multi-targets and their potential mechanisms of action were also explored, which is a possible way to combine network pharmacology and traditional Chinese medicine methods as a treatment for AD.

Materials and methods

The flowchart of this study is shown in Figure 1.

Screening and target prediction of active ingredients in Wuling capsules

The Wuling capsules used in this study were produced with the approvement of State Medical Permitment (Z19990048). Since the active ingredients of Wuling capsules were not retrieved in TCMSP, TCMID, Batman, and other databases, we obtained useful ingredients from the literature of CNKI and Pubmed. We then entered the retrieved active ingredients into the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/) to obtain the two-dimensional molecular structure and Pubchem CID. Based on the SwissADME database (http://www.swissadme.ch/index.php), we screened the two-dimensional molecular formula of the active ingredient with gastrointestinal absorption as high and at least three terms of drug-likeness were yes. Then, based on the SwissTargetPrediction website (http://www.swisstargetprediction.ch/), the targets of the active ingredients of Xylaria nigripes powder were obtained.

Obtaining targets related to AD

After entering the keyword "Alzheimer's disease" in the GeneCards database (version 5.11, www.genecards.org), 1305 disease targets related to AD were obtained and Venn diagrams were drawn.

Obtaining common targets of diseases and drugs

Based on the Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/), we intersected the potential targets of the chemical constituents of Wuling capsules with AD-related disease targets and drew a Venn diagram.

Construction of the drug-ingredient-target-disease network

After obtaining the key targets of Wuling capsule against AD, we constructed the drug-ingredient-target-disease network using Cytoscape software (version 3.9.1). Circles of different colors represent drugs, active ingredients, targets, and AD, respectively; at the same time, a straight line indicates their interconnectedness.

Gene Ontology (GO) enrichment analysis and Kyoko Encyclopedia of Genes and Genomes (KEGG) pathway analysis

To further understand the biological processes and related pathways of these key targets, we performed GO enrichment analysis and KEGG pathway analysis. First, we entered these targets into the online analysis website Metascape (https://metascape.org/gp/index.html#/main/step1) and obtained the results of GO and KEGG. Then, we visualized these results through another online analysis website Bioinformatics (http://www.bioinformatics.com.cn/). In the meantime, we constructed the top 10 pathway-target network by Cytoscape software (version 3.9.1).

Construction of the PPI network of core targets of Wuling capsule-AD

To further explore the connections between the various proteins, disease, and drug, intersection targets were entered into the STRING database (version 11.5, https://cn.string-db.org/) and the species selected was "*Homo sapiens*" in the organism column, which were used to search for known and predicted PPIs. In the "Setting" section of the database, free nodes were hidden and a confidence score of ≥0.4 was set to obtain the PPI analysis results for core targets. Next, the acquired data were imported into Cytoscape software (version 3.9.0) to construct the PPI network. Using the Cytoscape software's internal function Analyze Network, we obtained a visual network graph based on the degree value. In the "Filter" module, we set all the target points as circles of different sizes and colors according to the size of the degree value and arrange them into six concentric circles. Finally, the topology parameters were calculated by the CytoHubba function in Cytoscape 3.9.0, including each node degree, the betweenness centrality, closeness centrality, and average shortest path length, all of which provide insight into the interaction network precise analysis of node properties by using topological analysis. The 20 targets were obtained and constructed as a PPI network of core targets based on the degree value.

Network construction of MCODE functional modules of potential protein functions

In addition to the PPI network of the above core targets, we inputted the intersection targets into the Metascape website (https://metascape.org/gp/index.html#/main/step) to obtain potential protein functional modules through the MCODE calculation method. According to the *p*-value, the biological processes with the three best scores were retained for functional analysis.

Results

Active ingredients and potential targets of Wuling capsules

After database and literature searches, we obtained a total of 138 active ingredients. The main ingredient of the Wuling capsule is Xylaria nigripes powder. Duplicate values and ingredients that did not meet the conditions of the SwissADME system were removed, and 54 active ingredients were included. Among Xylaria nigripes, the amino acid class accounted for 27.5% of the total, such as serine, proline, leucine, etc. Meanwhile, leucine corresponded to the largest number of targets. Isocoumarins are important constituents of Wuling capsules, including 5-carboxymellein, 5-methylmellein, genistein, etc. Quercetin is also an essential active ingredient in the efficacy of Wuling capsules for the treatment of AD.[8] The abbreviation, molecular name, numbers of targets, and Pubchem CID of Wuling capsule active compounds are shown in Table 1. These active compounds involve 943 targets in addition to repeat targets.

Targets related to AD

By entering the keyword "Alzheimer" into the GeneCards database, we obtained 11,126 targets of AD. We then set the relevance score to greater than three times the median and set these eligible targets as AD disease targets. The maximum relevance score of the targets obtained by the GeneCards database was 108.06, and the minimum was 0.07. The median of the first calculation was 0.44, the median of the second calculation was 0.82, the median of the third calculation was 1.37, and the final target with a correlation score ≥1.37 was set as the disease target for AD.

Potential targets of Wuling capsule in the treatment of AD

Through literature and database searches, 943 potential targets of active ingredients were identified. By searching the Genecard database, we obtained a total of 1305 AD-related targets after removing duplicates. In this study, 284 common targets related to Wuling capsule and AD were retrieved and obtained through the Draw Venn diagram website (Fig. 2), and those could be used as potential targets of Wuling capsule for AD treatment as well as for the following pathway enrichment analysis and PPI network construction.

Table 1. Ingredients and target information of Wuling capsules

Abbreviation	Pubchem CID	Molecular name	Number of targets
WLJ-1	119085540	5-Ethoxymethyl-1H-pyrrole-2-carbaldehyde	100
WLJ-2	14807789	5-Methylmellein	75
WLJ-3	6441391	Curdione	58
WLJ-4	5280961	Genistein	62
WLJ-5	190	Adenine	19
WLJ-6	5960	Aspartic acid	36
WLJ-7	33032	Glutamate	32
WLJ-8	119	Gamma-aminobutyric acid	3
WLJ-9	5962	Lysine	18
WLJ-10	5951	Serine	28
WLJ-11	145742	Proline	106
WLJ-12	750	Glycine	3
WLJ-13	6287	Valine	103
WLJ-14	6137	Methionine	89
WLJ-15	6306	Isoleucine	60
WLJ-16	6106	Leucine	164
WLJ-17	6274	Histidine	102
WLJ-18	619721	2-Hydrazino-8-hydroxy-4-phenylquinoline	103
WLJ-19	16251	3,4-Dimethoxyphenol	102
WLJ-20	7311	2,4-Bis(1,1-dimethylethyl)phenol	104
WLJ-21	442027	Ferruginol	102
WLJ-22	135	Hydroxybenzoic acid	100
WLJ-23	445858	Ferulic acid	101
WLJ-24	5280343	Quercetin	102
WLJ-25	1174	Uracil	73
WLJ-26	6305	Tryptophan	100
WLJ-27	46217964	Xylarenolide	103
WLJ-28	46217965	Xylaranol A	107
WLJ-29	46217966	Xylaranol B	102
WLJ-30	46217967	Xylaranic acid	100
WLJ-31	16091621	Mairetolide F	102
WLJ-32	101515910	Furofurandione	107
WLJ-33	139583529	Xylaric acid A	100
WLJ-34	139583562	Xylaric acid B	100
WLJ-35	139587443	Xylaric acid C	100
WLJ-36	38354723	Xylaric acid D	100
WLJ-37	74015886	Trichoderonic acid A	100
WLJ-38	71467176	Helicascolide C	128
WLJ-39	6439087	Helicascolide A	102
WLJ-40	46832764	Xylarioic acid B	100

(continued)

Table 1. *(continued)*

Abbreviation	Pubchem CID	Molecular name	Number of targets
WLJ-41	46832768	Methyl xylariate C	105
WLJ-42	46832769	Xylariolide D	102
WLJ-43	23872095	6-[(*1R*)-1-Hydroxypentyl]-4-methoxy-2H-pyran-2-one	103
WLJ-44	78429	Dihydroisocoumarin	102
WLJ-45	486250	5-Carboxymellein	100
WLJ-46	182323	Tryptoquivaline L	101
WLJ-47	10544575	Sphaeropsidin C	100
WLJ-48	6476044	Piliformic acid	100
WLJ-49	637542	*p*-Coumaric acid	100
WLJ-50	370	Gallic acid	100
WLJ-51	8468	Vanillic acid	100
WLJ-52	689043	Caffeic acid	101
WLJ-53	5281855	Ellagic acid	104
WLJ-54	26495249	Gliocladic acid	100

Drug-ingredient-target-disease network

Wuling capsule only contains one drug Xylaria nigripes, and this drug has 54 active ingredients, which have 285 anti-AD targets. We visualized their relationships through the drug-ingredient-target-disease network shown in Figure 3. The green circle represents Xylaria nigripes, the orange circles represent active ingredients of Xylaria nigripes, and the blue and the red circles represent key targets and disease, respectively.

GO enrichment analysis and KEGG pathway analysis

As depicted in Figure 4, through the results of Metascape, we found that the main anti-AD targets were primarily involved in biological processes including cellular response to nitrogen compounds, behavior, synaptic signaling, regulation of the mitogen-activated protein kinase (MAPK) cascade and trans-synaptic signaling, etc.

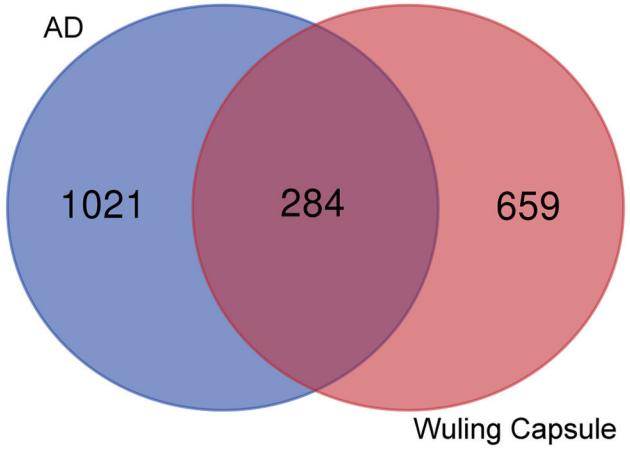

Fig. 2. Potential targets for Wuling capsules in the treatment of AD. The purple circles represent the targets of the Wuling capsule, the red circles represent the target in AD, and the intersecting part in the middle is the target of the Wuling capsule in the treatment of AD. AD, Alzheimer's disease.

The cellular components involved mainly include dendrites, dendritic tree, postsynapse, neuronal cell body, glutamatergic synapse, etc. The molecular functions of these targets are mainly enriched in protein kinase activity, phosphotransferase activity, alcohol group as an acceptor, neurotransmitter receptor activity, kinase activity, oxidoreductase activity, etc. According to the results of the KEGG pathway analysis, we found that these targets were mainly enriched in pathways of cancer, pathways of multiple neurodegenerative diseases, AD, the PI3K/AKT signaling pathway, apoptosis, etc. As shown in Figure 4, these targets are mainly associated with the pathogenesis of AD. Among the 20 pathways, we chose the top 10 and constructed the pathway-target network (Fig. 5) to visually observe the relationship between the major pathways and their involved targets. We found that the different pathways include the same targets and that different targets can be involved in the same pathways, further explaining the multiple mechanisms of Wuling capsule in the treatment of AD.

Construction of the PPI network based on topology analysis and acquisition of core targets

Through the GeneCards database, we screened according to the median and obtained 11,126 gene targets. To reveal the mechanism of action of the Wuling capsule on AD and to obtain potential genes more comprehensively and accurately, we inputted 284 intersection targets into the STRING database to obtain the PPI network. After deleting the scattered targets and setting a confidence score of ≥0.4, the analytical results of the STRING database were imported into Cytoscape software for visualization. Then, a PPI network with 283 nodes and 10,552 edges was constructed by the NetworkAnalyzer plugin in Cytoscape software. In this network, the average number of neighbors was 37,286, and the average node degree value was 74.57. According to the size of the degree value in the PPI network, the 284 intersection targets were arranged into six concentric circles from the outside to the inside. The larger the quantitative value of the parameter is, the closer the position is to the center of the circle shown in Figure 6a, which means that the nodes in the network are more important. Moreover, the PPI visualization network contains nodes of

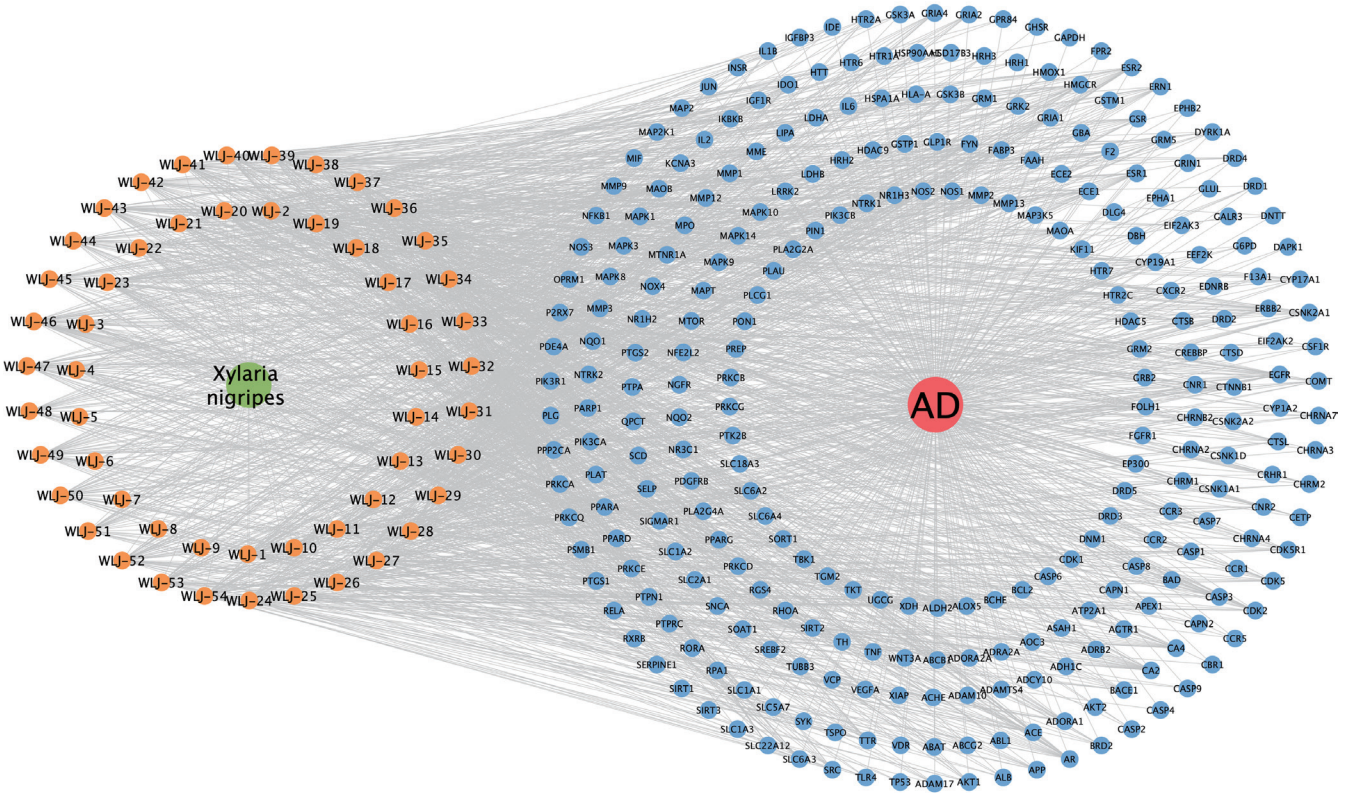

Fig. 3. The drug-ingredient-target-disease network. The green circles represent the drugs in the Wuling capsule, the orange circles represent the active ingredients in the Wuling capsule, the blue circles represent the targets of the Wuling capsule treating Alzheimer's disease, the red circles represent diseases, and the gray lines indicate their connections. AD, Alzheimer's disease.

different colors, and the darker the color, the more important it is. Next, a topological analysis of 284 potential genes was performed using CytoHubba, a plugin for Cytoscape, and a new network of key targets consisting of 20 nodes and 189 edges was proposed (Fig. 6b), including AKT, glyceraldehyde-3-phosphate dehydrogenase, tumor necrosis factor (TNF), serum albumin, tumor suppressor p53, interleukin (IL) 6, IL1β, MAPK3, caspase-3, proto-oncogene c-JUN, tyrosine-protein kinase sarcoma, vascular endothelial growth factor A, axin1/beta-catenin, epidermal growth factor receptor erbB1, heat shock protein 90-alpha, mammalian target of rapamycin (mTOR), beta-amyloid A4 protein, estrogen receptor alpha, peroxisome proliferator-activated receptor gamma, and cyclooxygenase 2 (COX2). Table 2 shows the information of the top 20 hub genes, such as the average shortest path length, betweenness centrality, closeness centrality, degree value, full name of the target, and Uniprot ID. Nodes with a high correlation have a great influence on the network, which may provide new ideas for the Wuling capsule to improve cognitive impairment in the future. These 20 Wuling capsule-AD-related genes were introduced into the Metascape system to carry out KEGG pathway enrichment, and the species was set as "*Homo sapiens*". The KEGG pathway results showed that there were 20 important potentially enriched pathways here, and they were ranked in ascending order of *p*-value ($p < 0.05$), including human cytomegalovirus infection ($n = 13$), pathways in cancer ($n = 14$), Kaposi sarcoma-associated herpesvirus infection ($n = 11$), proteoglycans in cancer ($n = 11$), lipid and atherosclerosis ($n = 11$), fluid shear stress and atherosclerosis ($n = 9$), AD ($n = 11$), Salmonella infection-related colorectal cancer ($n = 10$), IL17 signaling pathway ($n = 8$), endocrine resistance ($n = 8$), advanced glycation end products/receptor for advanced glycation end products signaling pathway in diabetic complications ($n = 8$), C-type lectin receptor signaling pathway ($n = 8$), TNF signaling pathway ($n = 8$), human papillomavirus infection ($n = 10$), chemical carcinogenesis-receptor activation ($n = 9$), shigellosis ($n = 9$), breast cancer ($n = 8$), epidermal growth factor receptor erbB1, tyrosine kinase inhibitor resistance ($n = 7$), and hepatitis B ($n = 7$). Interestingly, we found that several target genes were involved in multiple pathways. We imported the KEGG enrichment results into the bioinformatics website for analysis and visualization and obtained a new enrichment analysis bubble chart (Fig. 7). To further intersect the potential biological information of the targets, we calculated five potential protein functional modules (Fig. 8) using the MCODE plugin of the Metascape database and selected the three best-scoring biological processes. Finally, we imported them into Cytoscape software for modeling and presented the specific information in Table 3.

Discussion

Traditional Chinese medicine represents great development value and is often proposed for the prevention and treatment of dementia.[9] In addition, network pharmacology has recently been applied to traditional Chinese medicine research to treat AD, to obtain multiple targets, and to determine multi-channel treatment methods. This is the first study to use network pharmacology to investigate the efficacy and potential pharmacological mechanisms of

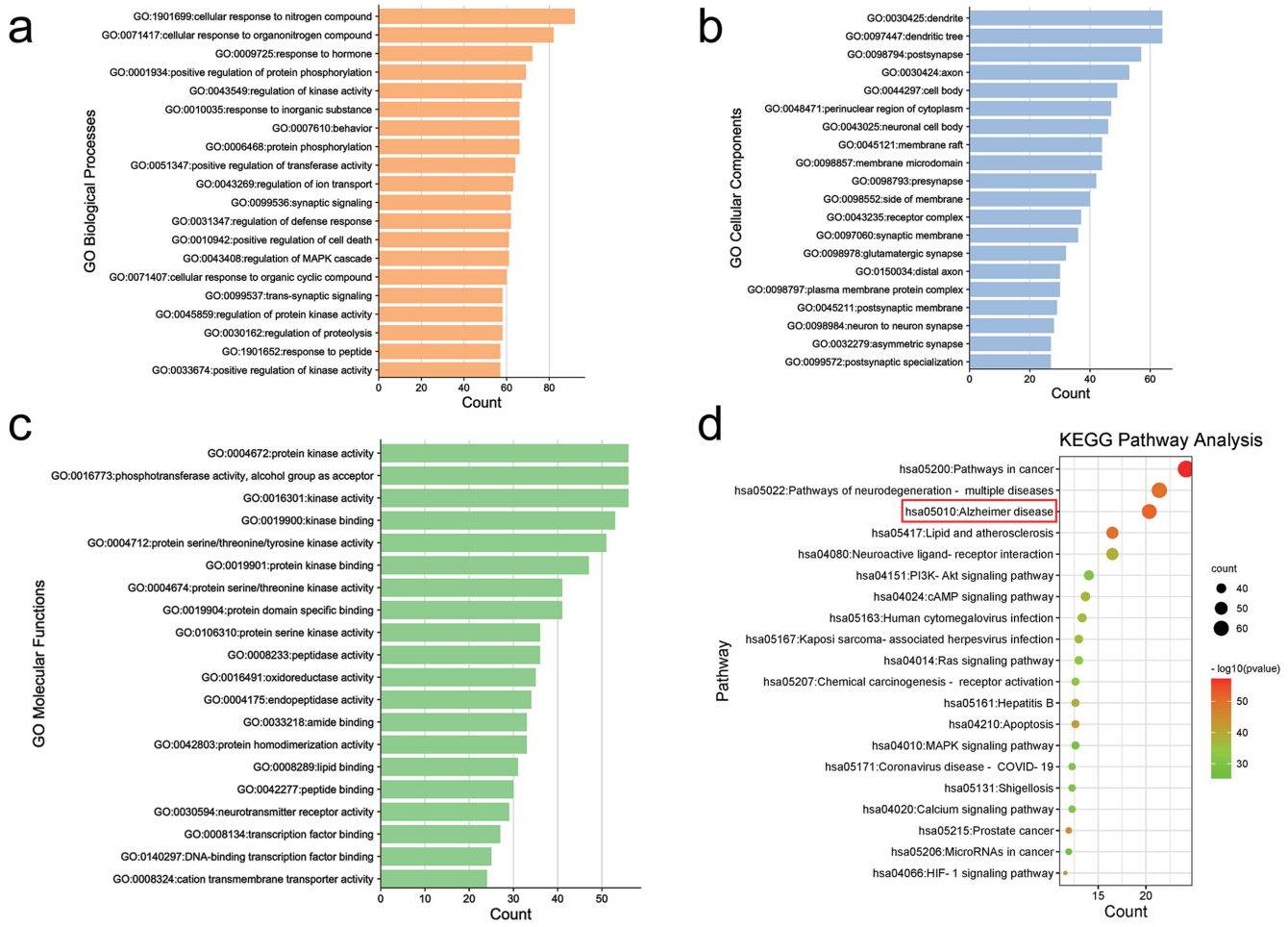

Fig. 4. Gene Ontology and Kyoko Encyclopedia of Genes and Genomes enrichment analysis of potential targets for Wuling capsule in the treatment of Alzheimer's disease.

Wuling capsules in the treatment of AD. According to the maximum relevance score of the targets, 1305 AD-related targets were screened. The network pharmacology results of the Wuling capsules identified 54 active ingredients, 284 target genes, and their main signaling pathways. The main active ingredients of Wuling capsules were found to be 5-ethoxymethyl-1H-pyrrole-2-carbaldehyde, quercetin, ellagic acid, xylarenolide, genistein, etc.

AD is a neurodegenerative disease with multiple pathological mechanisms. The main pathogenesis includes Aβ deposition in the brain, hyperphosphorylated tau, oxidative stress, and inflammation.[10] Studies have shown that the chemical constituents of Wuling capsules have neuroprotective effects, including anti-inflammatory and anti-oxidant properties.[11–13] Some clinical studies have proven that the addition of Wuling capsule treatment to conventional treatment for AD can reduce the inflammatory response, elevate the Aβ1-42 level, reduce the Tau protein and P-tau181 protein level, and then improve the cognitive function of patients, thus enhancing their daily living ability and quality of life.[14]

Quercetin is a flavonoid with anti-oxidant, hypolipidemic, and neuronal loss-inhibiting activities, which can be effective against AD via the MAPK pathway.[15,16] Studies have shown in a triple transgenic AD mouse model that oral quercetin prophylaxis is effective at reducing beta-amyloidosis and tends to reduce tauopathy in the hippocampus and amygdala.[17] In addition, it has been shown that quercetin can reduce the expression of inflammatory factors (e.g., IL6, TNFα) to inhibit neuroinflammation in AD.[18] Moreover, ellagic acid is an important active ingredient of Wuling capsule that has anti-oxidant and mitochondrial protective effects.[19,20] In pathological conditions, excessive activation of polyadenosine diphosphate ribose polymerase-1 leads to neuronal death.[21] Additionally, Khan *et al*. have demonstrated experimentally that quercetin has an anti-apoptotic effect on the mitochondria and has been shown to inhibit mitochondrial apoptosis in the mouse cerebral cortex and hippocampus by modulating Bax/Bcl2, thus reducing activated cytochrome c and caspase-3 activity as well as cleaving polyadenosine diphosphate ribose polymerase-1.[22] Moreover, an AD rat model experiment has demonstrated that ellagic acid attenuates oxidative stress and acetylcholinesterase activity as well as modulates the nuclear factor-kappaB/nuclear factor-erythroid factor 2-related factor 2/toll-like receptor 4 signaling pathways, thereby dose-dependently improving learning and memory impairment.[23] Based on the results of experimental studies, ellagic acid can activate the PI3K/AKT pathway as well as inhibit MAPK expression in AD.[19] Besides, ellagic acid can also reduce the inflammatory response in the AD brain by decreasing Aβ-stimulated TNFα secretion from microglia in mice.[24] Genistein can also scavenge

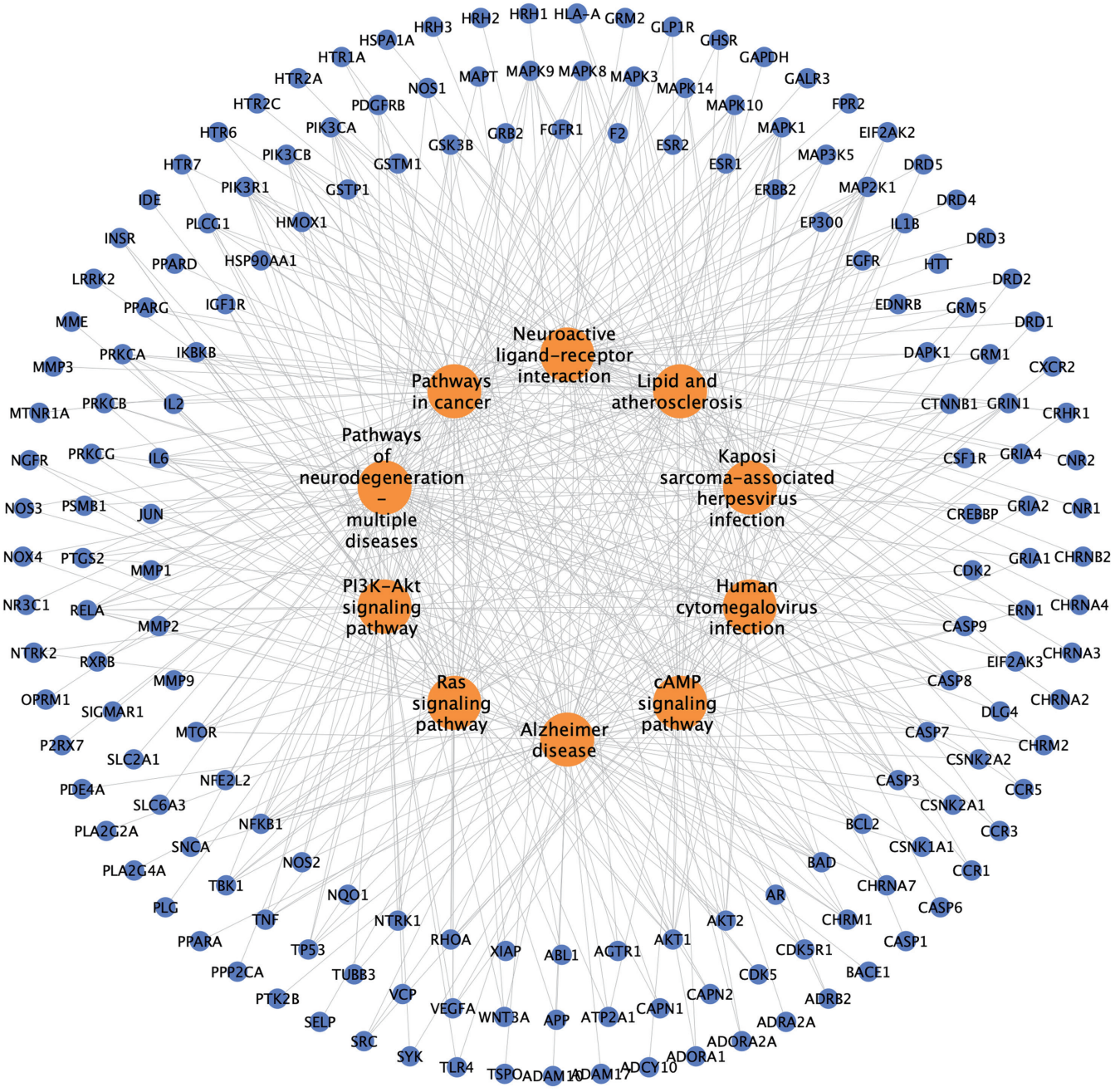

Fig. 5. The pathway-target network. Orange circles indicate pathways involved by potential targets, blue circles are targets, and gray lines indicate their connections.

reactive oxygen radicals in the AD brain through a PI3K/AKT/nuclear factor-erythroid factor 2-related factor 2 pathway-related mechanism to achieve an anti-oxidant effect.[25–27] Furthermore, in AD, genistein can mediate and activate the increased expression of peroxisome proliferator activated receptors in astrocytes, thereby suppressing various inflammatory responses induced by Aβ in primary cultured astrocytes.[28] Meanwhile, Song *et al*. have demonstrated that *p*-coumaric acid can protect against oxidative stress in a mouse model of hepatotoxicity.[8] As the main component of mannitol, β-glucans can regulate the immune response by inhibiting the inflammatory factors inducible nitric oxide synthase, COX2, and TNFα in macrophages.[29] In this study, through enrichment analysis of multiple targets, we found that it can also act on the modulation of chemical synaptic transmission. Experiments have shown that Wuling capsules can modulate the PI3K/AKT/mTOR pathway in the hippocampus to regulate the levels of neurotransmitters such as norepinephrine, dopamine, and serotonin.[30]

According to the KEGG pathway analysis, it was found that the targets of the Wuling capsule in the treatment of AD were mainly enriched in the pathways related to neurodegenerative diseases

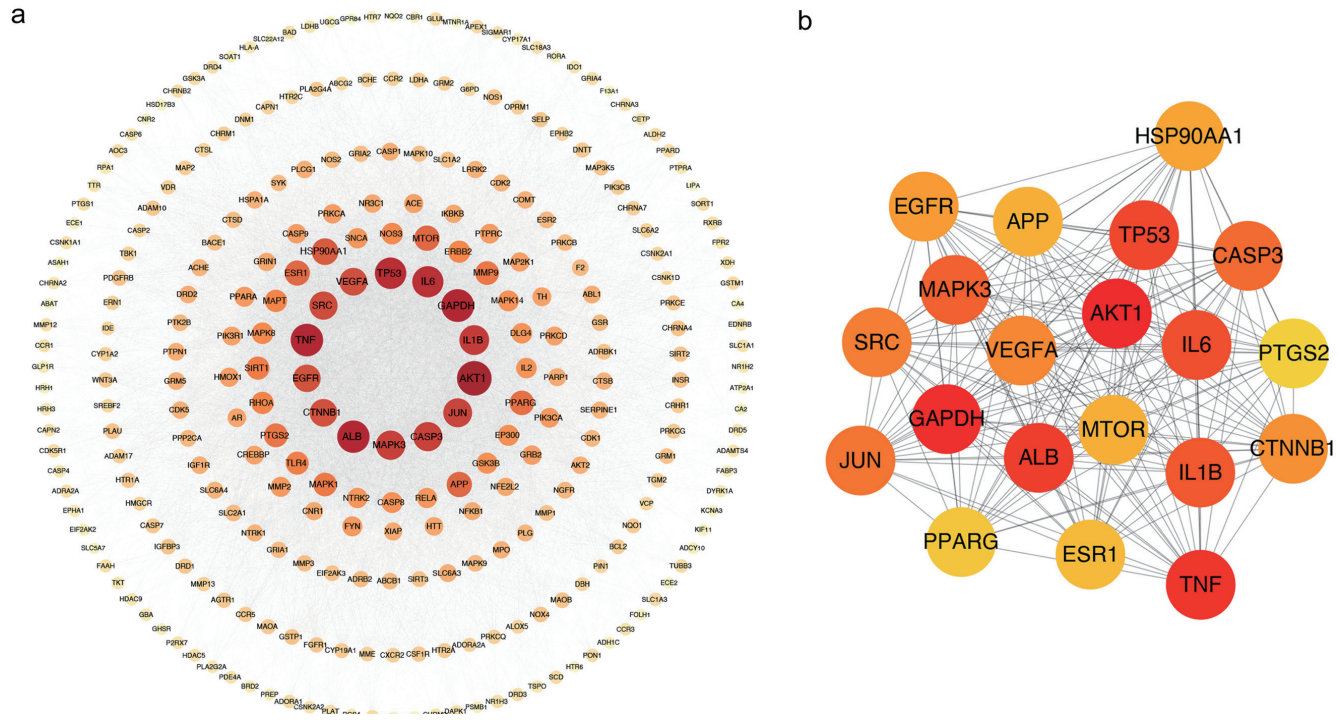

Fig. 6. The protein-protein interaction network of potential targets.

Table 2. Topological analysis of the top 20 key targets of the Wuling capsule-Alzheimer's disease network

No.	Target name	Abbreviation	Uniprot ID	ASPL	BC	CC	Degree
1	Serine/threonine-protein kinase B	AKT1	P31749	1.41	0.06	0.71	332
2	Glyceraldehyde-3-phosphate dehydrogenase, liver	GAPDH	P04406	1.46	0.04	0.69	308
3	Tumor necrosis factor-alpha	TNFα	P01375	1.47	0.04	0.68	304
4	Serum albumin	ALB	Q56G89	1.46	0.05	0.68	302
5	Tumor suppressor p53/oncoprotein Mdm2	TP53	P04637	1.51	0.04	0.66	290
6	Interleukin-6	IL6	P05231	1.50	0.03	0.67	286
7	Interleukin-1 beta	IL1B	P01584	1.54	0.03	0.65	266
8	Mitogen-activated protein kinase ERK1	MAPK3	P27361	1.56	0.02	0.64	260
9	Caspase-3	CASP3	P42574	1.55	0.02	0.65	258
10	Proto-oncogene c-JUN	JUN	Q6FHK0	1.57	0.02	0.64	254
11	Tyrosine-protein kinase sarcoma	SRC	P12931	1.59	0.02	0.63	244
12	Vascular endothelial growth factor A	VEGFA	P15692	1.60	0.01	0.63	238
13	Axin1/beta-catenin	CTNNB1	P35222	1.60	0.02	0.63	236
14	Epidermal growth factor receptor erbB1	EGFR	P00533	1.61	0.02	0.62	234
15	Heat shock protein 90-alpha	HSP90AA1	A0A0U1RR69		0.01	0.61	222
16	Serine/threonine-protein kinase mammalian target of rapamycin	mTOR	P42345	1.66	0.01	0.60	204
17	Beta amyloid A4 protein	APP	P05067	1.65	0.03	0.61	204
18	Estrogen receptor alpha	ESR1	P03372	1.66	0.02	0.60	202
19	Peroxisome proliferator-activated receptor gamma	PPARG	P37231	1.69	0.02	0.59	192
20	Cyclooxygenase-2	PTGS2	P35354	1.70	0.01	0.59	184

ASPL, average shortest path length; BC, betweenness centrality; CC, closeness centrality.

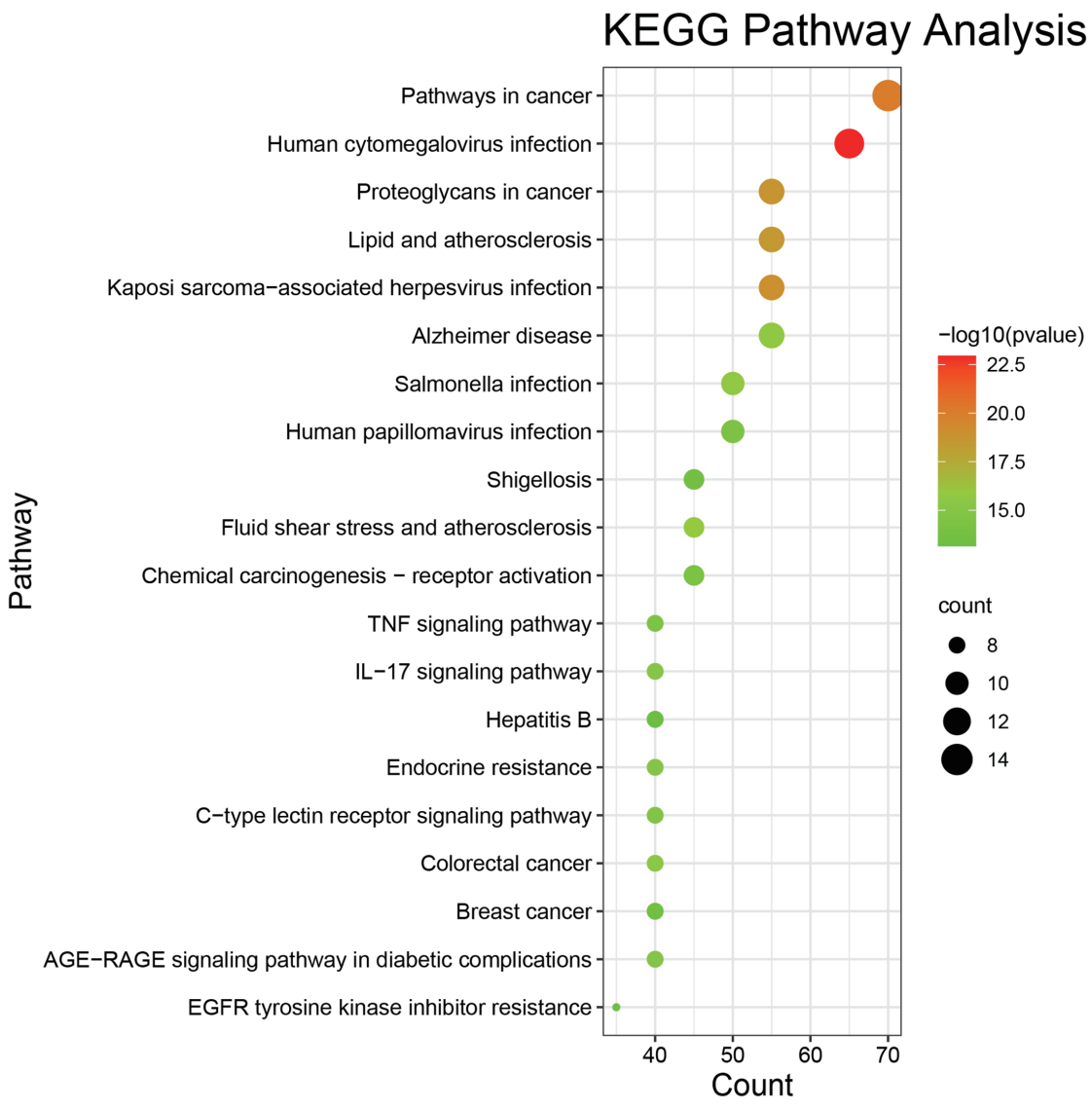

Fig. 7. Kyoko Encyclopedia of Genes and Genomes enrichment analysis of key targets for Wuling capsule in the treatment of Alzheimer's disease.

and ranked third in the pathway analysis results in AD, indicating that the Wuling capsule can treat AD to a certain extent. Moreover, these targets are also enriched in the PI3K/AKT signaling pathway. Studies also have found that the abnormality of the PI3K/AKT signaling pathway is important for the pathogenesis of AD.[31] Wuling capsule may improve the abnormal PI3K/AKT signaling pathway under the pathological conditions of AD; therefore, it may be able to treat AD. In the analysis of cellular components, we found that these key targets are mainly concentrated in the components related to synapses, and the damage of a large number of neurons and synapses in the AD brain is a pathological hallmark of AD.[32] The application of the Wuling capsule may improve the damaged synapses in the brain and re-establish the normal material information transmission system in the brain, thereby treating AD. Protein kinases are enzymes that catalyze the process of protein phosphorylation, and the phosphorylation of proteins is the last link in the transmission of neural information in cells, resulting in changes in the state of ion channel proteins and channel gates. Therefore, protein kinases are related to the transmission of neural information in the brain.[33] At the same time, protein kinases are also involved in the inflammatory process, and inflammation also has been shown to be one of the important pathological mechanisms of AD.[33] According to the molecular function analysis, it was found that the targets of the Wuling capsule for AD treatment were mainly focused on the functions related to protein kinases, indicating that the Wuling capsule may improve the neural information transmission process as well as the neurological processes and inflammatory state in the brain by regulating the activity of protein kinases, thereby treating AD. Although the top result in the biological processes results was the cellular response to nitrogen compounds, it can be seen in the latter results that these targets are involved in biological processes mainly related to synaptic signaling, regulation of ion transport, and positive regulation of cell death, indicating that Wuling capsule can improve the pathological state of AD and treat AD by regulating cell death and synaptic function.

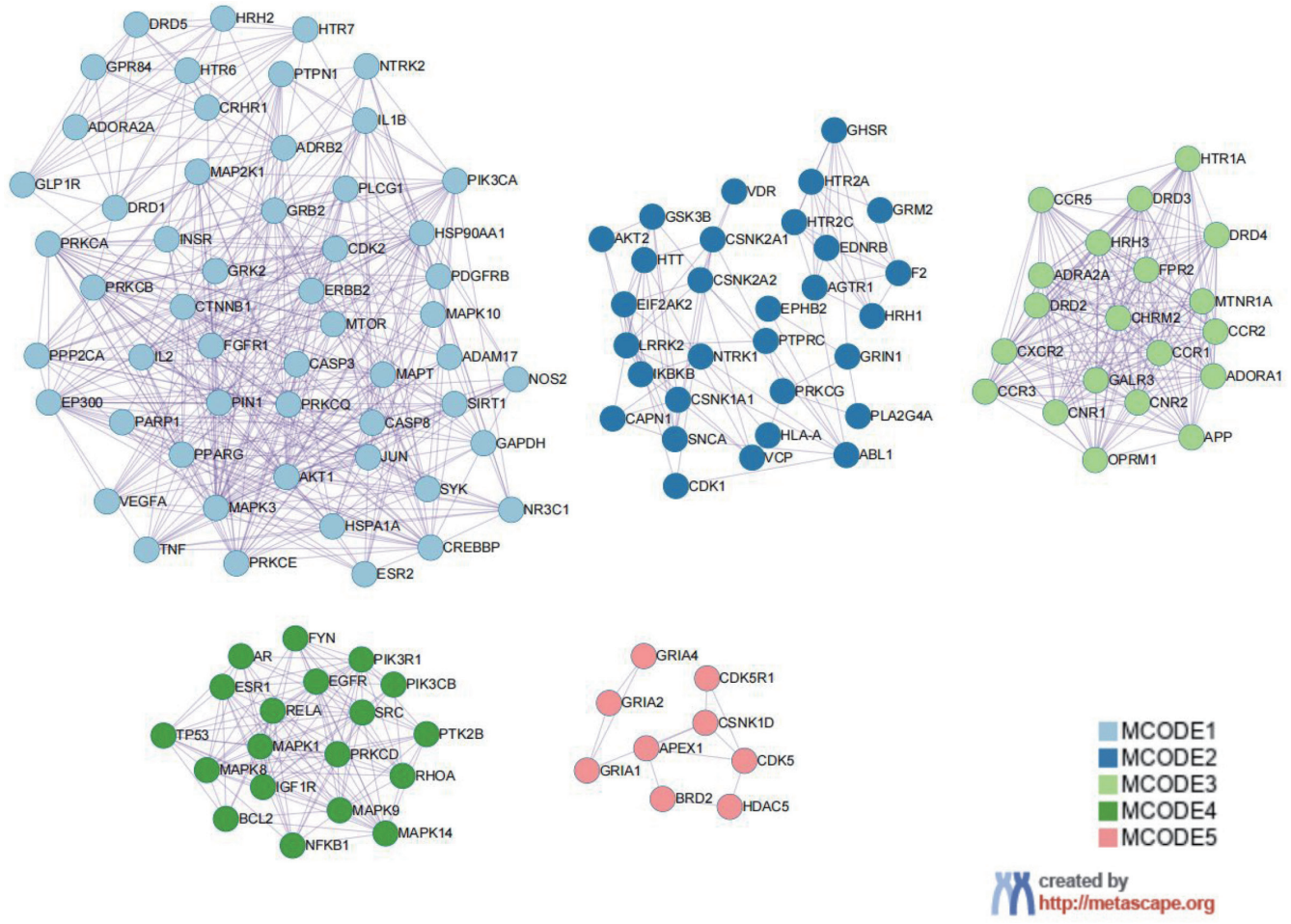

Fig. 8. Five potential protein functional modules.

Table 3. The functional description of potential MCODE networks

No.	MCODE	GO	Description	Log10(*p*)
1	MCODE_1	hsa05200	Pathways in cancer	−30.8
2	MCODE_1	GO:0051347	Positive regulation of transferase activity	−28.2
3	MCODE_1	WP4659	Gastrin signaling pathway	−26
4	MCODE_2	GO:0007610	Behavior	−14.5
5	MCODE_2	GO:0050804	modulation of chemical synaptic transmission	−14.3
6	MCODE_2	GO:0099177	regulation of trans-synaptic signaling	−14.3
7	MCODE_3	R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	−39.3
8	MCODE_3	WP455	G-protein coupled receptors, class A rhodopsin-like	−38.3
9	MCODE_3	R-HSA-500792	G-protein coupled receptors ligand binding	−36.4
10	MCODE_4	hsa01522	Endocrine resistance	−25.5
11	MCODE_4	hsa05131	Shigellosis	−25.3
12	MCODE_4	WP2374	Oncostatin M signaling pathway	−24.8
13	MCODE_5	GO:0035235	Ionotropic glutamate receptor signaling pathway	−10.3
14	MCODE_5	GO:1990806	ligand-gated ion channel signaling pathway	−10.3
15	MCODE_5	R-HSA-399710	Activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors	−10.1

In summary, the Wuling capsule can improve the pathological state of AD by regulating the PI3K/AKT signaling pathway and improving synaptic disorders, thereby achieving the purpose of treating AD.

The above-mentioned intersection targets of drugs and diseases obtained from the database are likely to be potential targets of Wuling capsules in the treatment of AD. Next, in order to further explore the core targets and biological processes related to Wuling capsule-AD, we used the PPI network and topology analysis to perform correlation analysis on 284 intersecting targets. Screening according to topological parameters demonstrated 20 key hub genes in the PPI network (Table 2). MAPK is phosphorylated under the stimulation of external stimuli such as neurotransmitters, cytokines, hormones, etc., which can regulate the growth of body cells and inflammatory responses. In other studies, MAPK3 and the proto-oncogene c-JUN have been identified as key therapeutic targets in AD.[34] To further elucidate the multiple mechanisms of action of Wuling capsule in the treatment of AD, KEGG enrichment analysis was performed on key targets, and 20 potential pathways were obtained. Pathways associated with AD involved 11 targets, such as AKT1, beta-amyloid A4 protein, caspase-3, axin1/beta-catenin, mTOR, glyceraldehyde-3-phosphate dehydrogenase, IL1β, IL6, MAPK3, COX2, and TNF. Studies have shown that the macro-autophagy/autophagy-lysosomal pathway plays an important role in AD pathogenesis and that the AKT/MAPK1/mTORC1 pathway can reduce its damage.[35] According to our KEGG enrichment analysis results, the PI3K/AKT pathway, a canonical pathway in AD pathogenesis, also was shown to be involved. In addition, Wang *et al*. used the familial AD mouse model with five mutations to demonstrate that the PI3K/AKT pathway plays an important role in reducing inflammatory responses and regulating M1-type microglia.[36] Moreover, the release of pro-inflammatory factors, such as IL1β, IL6, and TNFα, is closely related to aging and cognitive impairment.[37] Furthermore, pro-inflammatory factors in the blood crossing the blood-brain barrier causes activation of microglia and glial cells, which induces Aβ aggregation.[38] Vascular endothelial growth factor is also a target closely related to vascular integrity, and its overexpression is a common cause of an impaired blood-brain barrier.[39] In related experimental studies, the inflammatory factor COX2 has been associated with microglial activation in AD.[40] To gain a more comprehensive understanding of the relationships among the 284 key targets in the PPI network, we further analyzed the results of the MCODE plugin. The regulation of trans-synaptic signaling is associated with synaptic signaling and induces immunodeficiency and cognitive impairment. Studies have shown that inhibition of Aβ-induced pathological mechanisms can attenuate cognitive impairment by targeting genes for trans-synaptic signaling.[41] However, the above is only an analysis based on the database. Next, pharmacological experiments are needed to verify the mechanism of the Wuling capsule in the treatment of AD.

Conclusion

The therapeutic effect of traditional Chinese medicine combined with network pharmacology on diseases has been widely confirmed. In this study, we screened 54 active ingredients, 284 intersection targets, and 20 common targets through network computing, database query, and other methods, which demonstrated that synaptic signaling, the inflammatory response, neurotransmitter transmission, neuronal loss, and other pathways are involved in the effects of Wuling capsule on AD. Thus, Wuling capsule shows promise as a multi-component, multi-target, and multi-path treatment of AD.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

Study concept and design (TM); analysis and interpretation of data, figure preparation, and writing of the manuscript text (HNY, GHH); acquisition of data (HNY, GHH, MJS); critical revision of the manuscript for important intellectual content and procurement of funding (TM). All authors reviewed the manuscript and approved the version to be published.

Data availability

The datasets used or analyzed during the present study are available from the corresponding author upon reasonable request.

References

[1] Peineau S, Rabiant K, Pierrefiche O, Potier B. Synaptic plasticity modulation by circulating peptides and metaplasticity: Involvement in Alzheimer's disease. Pharmacol Res 2018;130:385–401. doi:10.1016/j.phrs.2018.01.018, PMID:29425728.

[2] Zhang XX, Tian Y, Wang ZT, Ma YH, Tan L, Yu JT. The Epidemiology of Alzheimer's Disease Modifiable Risk Factors and Prevention. J Prev Alzheimers Dis 2021;8(3):313–321. doi:10.14283/jpad.2021.15, PMID:34101789.

[3] Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, *et al*. Alzheimer's disease. The Lancet 2021;397(10284):1577–1590. doi:10.1016/s0140-6736(20)32205-4, PMID:33667416.

[4] Gong QF, Wu SH, Tan NH, Chen ZH. Study on compounds with antioxidant and antitumor activity from fermental mycelium of Xylaria nigripes. Food Sci Technol 2008;33(12):28–31. doi:10.13684/j.cnki.spkj.2008.12.067.

[5] He XD, Zhang LS, Chen JF, Hu XY, Chen W. Effects of Wuling Myceliaon Seizure Development and Memory Impairment Induced by Pentylenetetrazole Kindling Epilepsy in Rats. Chin Pharm J 2010;45(16):1238–1242.

[6] Yang N, Hao W, Liu Y, Ji C, Zuo P. Behavioral studies on the anxiolytic effects of Xylaria nigripes. Chinese Journal of Ethnomedicine and Ethnopharmacy 2010;19(5):27–28,30. doi:10.3969/j.issn.1007-8517.2010.05.019.

[7] Zou XD, Zhang ZZ, Cheng YG, Li ZC, Wang H, Hu ZW. Effect of Wuling Capsule on cognitive function of mice. Chin J Clin Pharmacol 2018;20:2421–2423. doi:10.13699/j.cnki.1001-6821.2018.20.008.

[8] Song A, Ko HJ, Lai MN, Ng LT. Protective effects of Wu-Ling-Shen (Xylaria nigripes) on carbon tetrachloride-induced hepatotoxicity in mice. Immunopharmacol Immunotoxicol 2011;33(3):454–460. doi:10.3109/08923973.2010.534100, PMID:21108581.

[9] Jarrell JT, Gao L, Cohen DS, Huang X. Network Medicine for Alzheimer's Disease and Traditional Chinese Medicine. Molecules 2018;23(5):1143. doi:10.3390/molecules23051143, PMID:29751596.

[10] Wang H, Zou XD, Yang QQ, Zhang YL, Guo DJ. Effect of Wu-Ling Powder therapy on cognition,Aβ1-42 expression in APPswe /PS1dE9 and p-Tau double transgenic AD mice. Chin J General Practice 2019;8:1279–1281.
[11] Jen CI, Su CH, Lu MK, Lai MN, Ng LT. Synergistic anti-inflammatory effects of different polysaccharide components from Xylaria nigripes. J Food Biochem 2021;45(4):e13694. doi:10.1111/jfbc.13694, PMID:33687093.
[12] Ko HJ, Song A, Lai MN, Ng LT. Immunomodulatory properties of Xylaria nigripes in peritoneal macrophage cells of Balb/c mice. J Ethnopharmacol 2011;138(3):762–768. doi:10.1016/j.jep.2011.10.022, PMID:22044578.
[13] Li J, Li LQ, Long HP, Liu J, Jiang YP, Xue Y, *et al*. Xylarinaps A-E, five pairs of naphthalenone derivatives with neuroprotective activities from Xylaria nigripes. Phytochemistry 2021;186:112729. doi:10.1016/j.phytochem.2021.112729, PMID:33721798.
[14] Fu YZ, Tong JF, Di LL, Zhang J, Liu XH. Clinical Observation of Wuling Capsules Combined with Oxiracetam in the Treatment of Alzheimer's Disease. China Pharmaceuticals 2021;(4):69–71. doi:10.3969/j.issn.1006-4931.2021.04.019.
[15] Zu G, Sun K, Li L, Zu X, Han T, Huang H. Mechanism of quercetin therapeutic targets for Alzheimer disease and type 2 diabetes mellitus. Sci Rep 2021;11(1):22959. doi:10.1038/s41598-021-02248-5, PMID:34824300.
[16] Khan H, Ullah H, Aschner M, Cheang WS, Akkol EK. Neuroprotective Effects of Quercetin in Alzheimer's Disease. Biomolecules 2019;10(1):59. doi:10.3390/biom10010059, PMID:31905923.
[17] Armstrong SA, Herr MJ. Preventive Effect of Quercetin in a Triple Transgenic Alzheimer's Disease Mice Model. StatPearls Publishing; 2022.
[18] Dash UC, Swain SK, Kanhar S, Banjare P, Roy PP, Dandapat J, *et al*. The modulatory role of prime identified compounds in Geophila repens in mitigating scopolamine-induced neurotoxicity in experimental rats of Alzheimer's disease via attenuation of cholinesterase, beta-secretase, MAPt levels and inhibition of oxidative stress imparts inflammation. J Ethnopharmacol 2022;282:114637. doi:10.1016/j.jep.2021.114637, PMID:34534598.
[19] Li HL, Zhang SY, Ren YS, Zhou JC, Zhou YX, Huang WZ, *et al*. Identification of ellagic acid and urolithins as natural inhibitors of Abeta25-35-induced neurotoxicity and the mechanism predication using network pharmacology analysis and molecular docking. Front Nutr 2022;9:966276. doi:10.3389/fnut.2022.966276, PMID:35983489.
[20] Ramadan WS, Alkarim S. Ellagic Acid Modulates the Amyloid Precursor Protein Gene via Superoxide Dismutase Regulation in the Entorhinal Cortex in an Experimental Alzheimer's Model. Cells 2021;10(12):3511. doi:10.3390/cells10123511, PMID:34944019.
[21] Chaitanya GV, Steven AJ, Babu PP. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. Cell Commun Signal 2010;8:31. doi:10.1186/1478-811X-8-31, PMID:21176168.
[22] Khan A, Ali T, Rehman SU, Khan MS, Alam SI, Ikram M, *et al*. Neuroprotective Effect of Quercetin Against the Detrimental Effects of LPS in the Adult Mouse Brain. Front Pharmacol 2018;9:1383. doi:10.3389/fphar.2018.01383, PMID:30618732.
[23] Kiasalari Z, Heydarifard R, Khalili M, Afshin-Majd S, Baluchnejadmojarad T, Zahedi E, *et al*. Ellagic acid ameliorates learning and memory deficits in a rat model of Alzheimer's disease: an exploration of underlying mechanisms. Psychopharmacology (Berl) 2017;234(12):1841–1852. doi:10.1007/s00213-017-4589-6, PMID:28303372.
[24] Rojanathammanee L, Puig KL, Combs CK. Pomegranate polyphenols and extract inhibit nuclear factor of activated T-cell activity and microglial activation in vitro and in a transgenic mouse model of Alzheimer disease. J Nutr 2013;143(5):597–605. doi:10.3945/jn.112.169516, PMID:23468550.
[25] Devi KP, Shanmuganathan B, Manayi A, Nabavi SF, Nabavi SM. Molecular and Therapeutic Targets of Genistein in Alzheimer's Disease. Mol Neurobiol 2017;54(9):7028–7041. doi:10.1007/s12035-016-0215-6, PMID:27796744.
[26] Guo J, Yang G, He Y, Xu H, Fan H, An J, *et al*. Involvement of alpha-7nAChR in the Protective Effects of Genistein Against beta-Amyloid-Induced Oxidative Stress in Neurons via a PI3K/Akt/Nrf2 Pathway-Related Mechanism. Cell Mol Neurobiol 2021;41(2):377–393. doi:10.1007/s10571-020-01009-8, PMID:33215356.
[27] Ma WW, Hou CC, Zhou X, Yu HL, Xi YD, Ding J, *et al*. Genistein alleviates the mitochondria-targeted DNA damage induced by beta-amyloid peptides 25-35 in C6 glioma cells. Neurochem Res 2013;38(7):1315–1323. doi:10.1007/s11064-013-1019-y, PMID:23519932.
[28] Valles SL, Dolz-Gaiton P, Gambini J, Borras C, Lloret A, Pallardo FV, *et al*. Estradiol or genistein prevent Alzheimer's disease-associated inflammation correlating with an increase PPAR gamma expression in cultured astrocytes. Brain Res 2010;1312:138–144. doi:10.1016/j.brainres.2009.11.044, PMID:19948157.
[29] Chen CF, Su CH, Lai MN, Ng LT. Differences in water soluble non-digestible polysaccharides and anti-inflammatory activities of fruiting bodies from two cultivated Xylaria nigripes strains. Int J Biol Macromol 2018;116:728–734. doi:10.1016/j.ijbiomac.2018.05.047, PMID:29763701.
[30] Wei S, Wang YF, Li H, Liu Y. Effects of Wuling Capsule on PI3K/Akt/mTOR pathway and neurotransmitters in hippocampus of post-stroke depression rats. Chin J Modern Med 2021;(14):47–51. doi:10.3969/j.issn.1005-8982.2021.14.009.
[31] Zheng J, Xie Y, Ren L, Qi L, Wu L, Pan X, *et al*. GLP-1 improves the supportive ability of astrocytes to neurons by promoting aerobic glycolysis in Alzheimer's disease. Mol Metab 2021;47:101180. doi:10.1016/j.molmet.2021.101180, PMID:33556642.
[32] Reddy PH, Oliver DM. Amyloid Beta and Phosphorylated Tau-Induced Defective Autophagy and Mitophagy in Alzheimer's Disease. Cells 2019;8(5):488. doi:10.3390/cells8050488, PMID:31121890.
[33] Armstrong SA, Herr MJ. Physiology, Nociception. StatPearls Publishing; 2022.
[34] Zeng P, Su HF, Ye CY, Qiu SW, Tian Q. Therapeutic Mechanism and Key Alkaloids of Uncaria rhynchophylla in Alzheimer's Disease From the Perspective of Pathophysiological Processes. Front Pharmacol 2021;12:806984. doi:10.3389/fphar.2021.806984, PMID:34975502.
[35] Zheng X, Lin W, Jiang Y, Lu K, Wei W, Huo Q, *et al*. Electroacupuncture ameliorates beta-amyloid pathology and cognitive impairment in Alzheimer disease via a novel mechanism involving activation of TFEB (transcription factor EB). Autophagy 2021;17(11):3833–3847. doi:10.1080/15548627.2021.1886720, PMID:33622188.
[36] Wang Y, Lin Y, Wang L, Zhan H, Luo X, Zeng Y, *et al*. TREM2 ameliorates neuroinflammatory response and cognitive impairment via PI3K/AKT/FoxO3a signaling pathway in Alzheimer's disease mice. Aging (Albany NY) 2020;12(20):20862–20879. doi:10.18632/aging.104104, PMID:33065553.
[37] Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, *et al*. IL-1beta, IL-6, TNF- alpha and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. Sci Rep 2018;8(1):12050. doi:10.1038/s41598-018-30487-6, PMID:30104698.
[38] Liu M, Xu Z, Wang L, Zhang L, Liu Y, Cao J, *et al*. Cottonseed oil alleviates ischemic stroke injury by inhibiting the inflammatory activation of microglia and astrocyte. J Neuroinflammation 2020;17(1):270. doi:10.1186/s12974-020-01946-7, PMID:32917229.
[39] Zhou Z, Gao S, Li Y, Peng R, Zheng Z, Wei W, *et al*. VEGI Improves Outcomes in the Early Phase of Experimental Traumatic Brain Injury. Neuroscience 2020;438:60–69. doi:10.1016/j.neuroscience.2020.04.042, PMID:32380270.
[40] Rangaraju S, Dammer EB, Raza SA, Rathakrishnan P, Xiao H, Gao T, *et al*. Identification and therapeutic modulation of a pro-inflammatory subset of disease-associated-microglia in Alzheimer's disease. Mol Neurodegener 2018;13(1):24. doi:10.1186/s13024-018-0254-8, PMID:29784049.
[41] Bouter Y, Kacprowski T, Rossler F, Jensen LR, Kuss AW, Bayer TA. miRNA Alterations Elicit Pathways Involved in Memory Decline and Synaptic Function in the Hippocampus of Aged Tg4-42 Mice. Front Neurosci 2020;14:580524. doi:10.3389/fnins.2020.580524, PMID:33013313.