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Network Pharmacology-Based Study on Intervention Mechanism of Lonicerae Japonicae Flos in the Treatment of Alzheimer's Disease

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Abstract:

The mechanism of Lonicerae Japonicae Flos in the treatment of Alzheimer's disease was analyzed by network pharmacology. The main active components and targets of Lonicerae Japonicae Flos are screened through TCMSP and online pharmacology database, the gene targets related to Alzheimer's disease are screened through genome annotation database, the target genes related to AD are screened through human Mendelian genetics (OMIM) database, and the intersection of drug and disease gene targets is obtained by venn diagram package of R language, The main effective components of honeysuckle were screened out for the treatment of Alzheimer's disease. The gene regulation network of Lonicerae Japonicae Flos for the treatment of AD was drawn by Cytoscape 3.7.2. The visual network diagram of gene protein interaction of honeysuckle was constructed by string database to screen the core genes, the GO and KEGG pathways of the screened gene targets were analyzed by R language. 17 main active components of Lonicerae Japonicae Flos were screened, and 158 gene targets may be involved in the treatment of AD. The results of go and KEGG pathway analysis show that honeysuckle treatment of AD mainly involves biological processes such as cytokine receptor binding, cytokine activity regulation and heme binding, regulating lipid and atherosclerosis, PI3K/Akt, human cytomegalovirus infection, Kaposi sarcoma associated herpesvirus infection Chemical carcinogenesis-receptor activation, hepatitis B, AGE-RAGE and other signaling pathways play a role. Through network pharmacology, the action characteristics of multi active components-multi targets-multi pathways of Lonicerae Japonicae Flos in the treatment of AD were constructed, and the gene targets and signal pathways of honeysuckle in the

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remedy of AD were comprehensively predicted, which provided a reference for the study of the mechanism of honeysuckle in the remedy of AD.

Keywords: *Lonicerae Japonicae Flos, Alzheimer's disease, TCMSP, OMIM.*

I. INTRODUCTION

Alzheimer's disease(AD) is an irreversible neurodegenerative disease caused by many reasons. It is the most common type of dementia. It is mainly characterized by progressive memory loss, cognitive impairment, inattention, affective disorder and personality change. Its incidence is closely related to age, genetic factors and environmental factors[1,2]. With the vigorous economic development, accelerated aging process and serious environmental pollution, the prevalence of AD has increased sharply. Drug treatment for AD has always been a research hotspot. Preclinical screening of relevant new drugs is being carried out on a large scale all over the world, but most of them end in failure. At present, only five kinds are approved for treatment, all of which are acetylcholinesterase inhibitors and glutamate receptor antagonists, but it can only slow down the symptoms and cannot change the disease process[3].

Lonicerae Japonicae Flos, a dried flower bud or newly opened flower of Lonicera japonica Thunb, a plant of the genus Lonicera in the Lonicerae Japonicae Flos family, is a common traditional Chinese medicine with a long history, which is mainly distributed in Henan, Shandong, Guangdong and other places[4]. Lonicerae Japonicae Flos is sweet in taste and cold in nature. It enters the heart, lung and stomach meridian. Its effect is mainly to clear away heat and detoxify. It is mainly used to treat febrile diseases, fever, carbuncle and gangrene toxin, heat toxin and blood dysentery. It has a variety of pharmacological effects and is widely used in clinical practice[5].The results showed that the aqueous extract of Lonicerae Japonicae Flos could prevent hippocampal amyloid β The accumulation of protein and the enhancement of insulin signal transduction in hippocampus decreased TNF-αand iNOS in hippocampal mice, prevent cognitive impairment and reverse fat oxidation[6]. Each dose group of Lonicerae Japonicae Flos leaf flavone can promote the increase of body mass, liver index, spleen index, heart index and brain index of mice to varying degrees, effectively enhance the physique of aging mice, and slow down the brain atrophy and the decline of organ function of mice[7].

Due to the complex and diverse components of Lonicerae Japonicae Flos, this study takes the pharmacodynamic components of Lonicerae Japonicae Flos with anti-ad effect as the research object, and analyzes the functional components, targets and mechanism of honeysuckle in the remedy of AD through network pharmacological methods, so as to provide more scientific evidence for the use of honeysuckle in the treatment of AD.

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II. EXPERIMENTAL METHOD

2.1 Screening of active components and potential targets of Lonicerae Japonicae Flos

Through TCMSP database[8] and related literature to obtain the chemical components and corresponding potential targets of walnut meal. Oral bioavailability(OB) reflects the proportion of drugs entering the systemic circulation after oral administration[9], and Drug likeness(DL) characterizes the characteristics of finished drugs according to the structure and properties of mixtures, which is used for preliminary screening in the early stage of drug discovery[10]. Generally, OB⩾30% and DL⩾0.18 are used as screening criteria.

2.2 Disease target collection

Using Genecards and OMIM of the two databases[11] searched for Alzheimer's disease related targets, merged the targets of the two databases to obtain AD targets, and collected Alzheimer's disease related targets with a score⩾3 as the screening condition.

2.3 Drugs – active ingredients – diseases – targets

The potential target of Lonicerae Japonicae Flos and the target protein related to AD were used in the draw Venn diagram is the intersection, which is the potential target of honeysuckles on Alzheimer's disease.

2.4 Network construction

Organize the intersection target information into excel format and import it into the network visualization software Cytoscape 3.7.2 construct the network diagram of "active ingredients - drugs-diseases-targets" of honeysuckle, and analyze the network topology parameters by using meta-analysis and network analyzer plug-in: select the top three active ingredients of degree as the main active ingredients[12], to further understand the interaction between active components of walnut meal and HLP related targets. At present, string(search tool for recurrent instances of neighboring genes) is the database with the most types of protein interaction[13]. The intersection targets are imported into string online analysis platform to obtain protein-protein interaction network (PPI). The confidence of protein-protein interaction score is set to 0.400, and non-interacting proteins are hidden. Export the obtained PPI data into TSV file format and import it into Cytospace 3.7.2 software to draw the network diagram, and analyze the network topology parameters by using the network analyzer plug-in.

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2.5 Go functional enrichment and KEGG signal pathway analysis

Install the Bioconductor package "org. HS. Eg.db" in R software and run it to convert the drug disease common target into entrezid. Then install the "clusterprofiler" package in the R software, according to the transformed entrezID, the functional enrichment analysis of key target genes GO and KEGG was performed at P<0.05 and q<0.05, and the results were output in the form of bar graph and bubble graph[14].

III. RESULT ANALYSIS

3.1 Screening results of active components from Lonicerae Japonicae Flos

Based on comprehensive database search and literature reports, 236 active components of Lonicerae Japonicae Flos were obtained from TCMSP database(Table 1). Among them, 23 components meet the OB ⩾ 30% and DL ⩾ 1.8, such as Ioniceracetalides B_qt(61.19,0.19), Centauroside_QT(55.79,0.50), caeruloside C(55.64,0.73), etc.

TABLE I Potential active ingredients of Lonicerae Japonicae Flos

Mol ID	Molecule Name	OB/%	DL
MOL003117	Ioniceracetalides B_qt	61.19	0.19
MOL001494	Mandenol	42	0.19
MOL001495	Ethyl linolenate	46.1	0.2
MOL003006	(-)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano[4,3-d]oxazolo[3,2-a]pyridine-3-carboxylic acid_qt	87.47	0.23
MOL000422	kaempferol	41.88	0.24
MOL002914	Eriodyctiol (flavanone)	41.35	0.24
MOL000006	luteolin	36.16	0.25
MOL003044	Chryseriol	35.85	0.27
MOL000098	quercetin	46.43	0.28
MOL003014	secologanic dibutylacetal_qt	53.65	0.29
MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	51.96	0.41
MOL003128	dinethylsecologanoside	48.46	0.48
MOL002707	phytofluene	43.18	0.50
MOL003111	Centauroside_qt	55.79	0.50
MOL003062	4,5'-Retro-.beta.,.beta.-Carotene-3,3'-dione, 4',5'-didehydro-	31.22	0.55
MOL003059	kryptoxanthin	47.25	0.57
MOL003101	7-epi-Vogeloside	46.13	0.58

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MOL002773	beta-carotene	37.18	0.58
MOL003124	XYLOSTOSIDINE	43.17	0.64
MOL003108	Caeruloside C	55.64	0.73
MOL000358	beta-sitosterol	36.91	0.75
MOL003036	ZINC03978781	43.83	0.76
MOL000449	Stigmasterol	43.83	0.76

3.2 Lonicerae Japonicae Flos is a potential target for Alzheimer's disease

Based on the 236 compounds obtained, a total of 1647 potential targets were searched. 12325 potential targets for AD were obtained from genecards and OMIM databases. 158 drug disease common targets were obtained by intersecting AD related genes with the potential targets of Honeysuckle through draw Venn diagram (Fig 1).

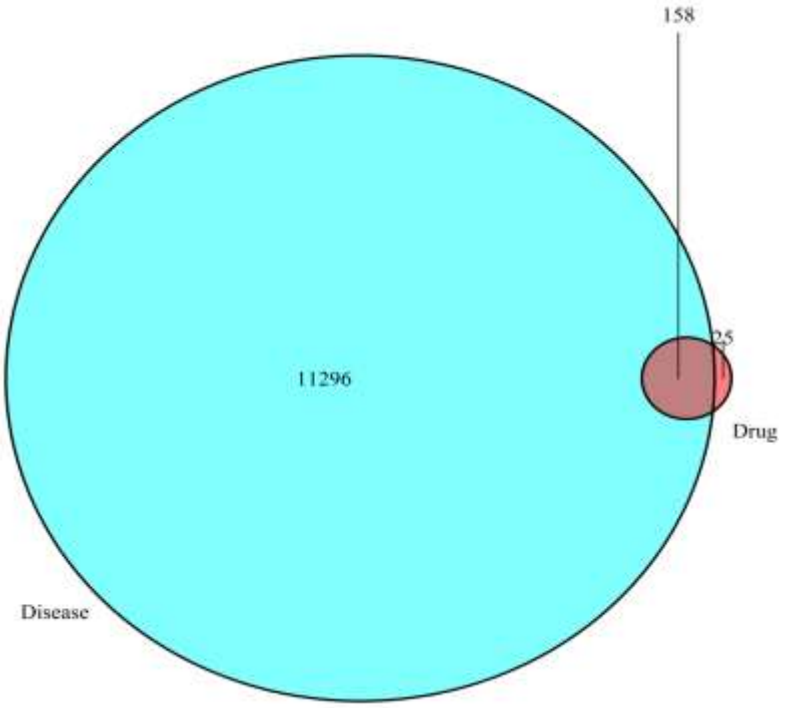

Fig 1: Gene regulation network of Lonicerae Japonicae Flos in the treatment of Schizophrenia

3.3 Network construction

The network diagram of "active component-drug-disease-target" of honeysuckle was constructed with Cytoscape3.7.2 software (Fig 3). The network diagram contains 177 nodes, 17 active ingredient and 158 action targets of honeysuckle. Kaempferol, Luteolin and Quercetin had the most targets. The targets of the most affected compounds were PTGS1, PTGS2, PPARG, PRSS1 and JUN. The 158 co-targets mentioned above were input into STRING database, and the PPI network of protein interaction was obtained by

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analysis (Fig 3A). Each edge represented the interaction between proteins, and the key proteins were AKT1, ALB, IL6, VEGFA, JUN, GASP3, etc. (Fig 3B). These proteins may play a key role in Honeysuckle's treatment of AD.

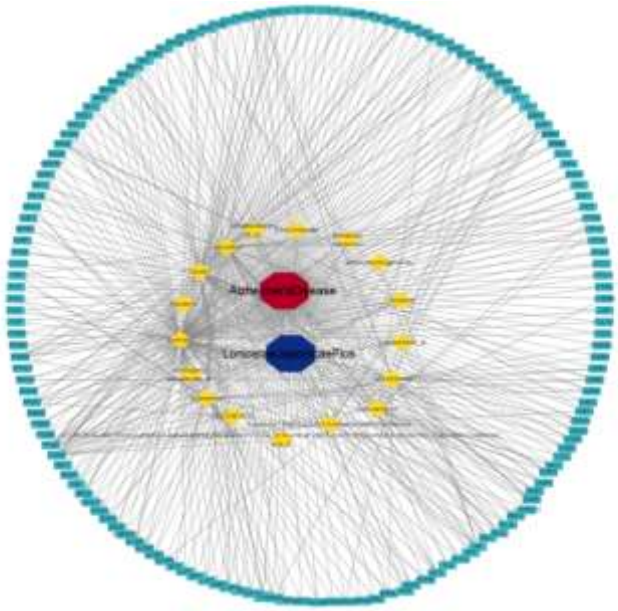

Fig 2: Active ingredient of Lonicerae Japonicae Flos -target network diagram

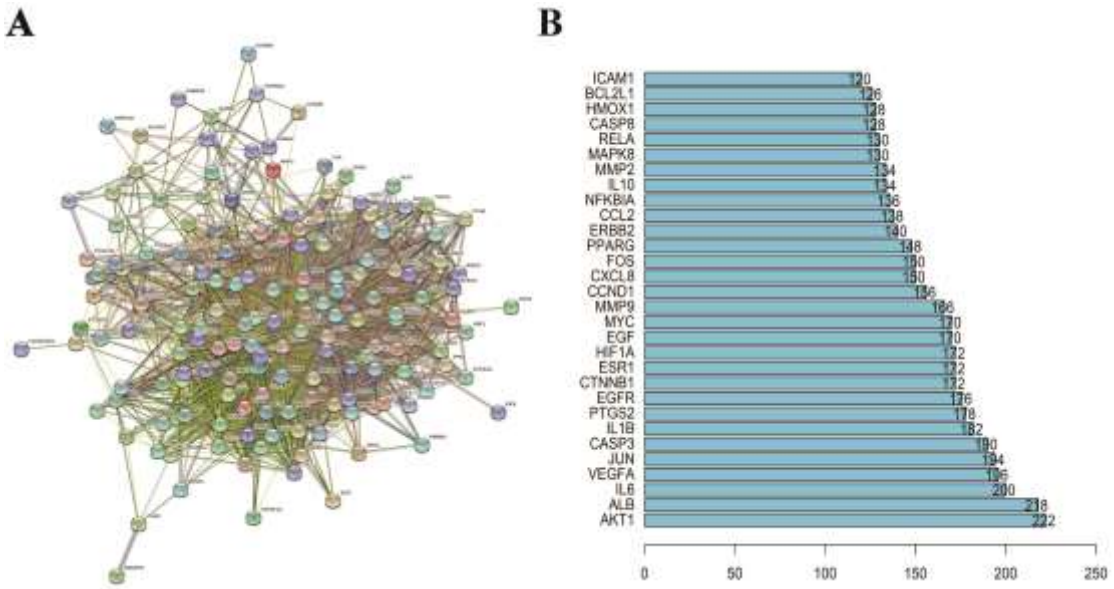

Fig 3: Protein interaction (PPI) network diagram (A) and key protein sequencing (B)

3.4 Go functional enrichment analysis

Go analysis is a directed acyclic diagram composed of the number of genes or proteins at a specific

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functional level, including molecular function, cell components and biological processes. According to the go analysis of this study, the top ranked are DNA binding transcription factor binding(26/158), RNA polymerase II specific DNA binding transcription factor binding(22/158), signaling receiver activator activity(18/158), ubiquitin like protein ligase binding(16/158), and protein heteromodulation activity(16/158), It shows that the relevant targets act on Alzheimer's disease by regulating different biological functions(Fig 4).

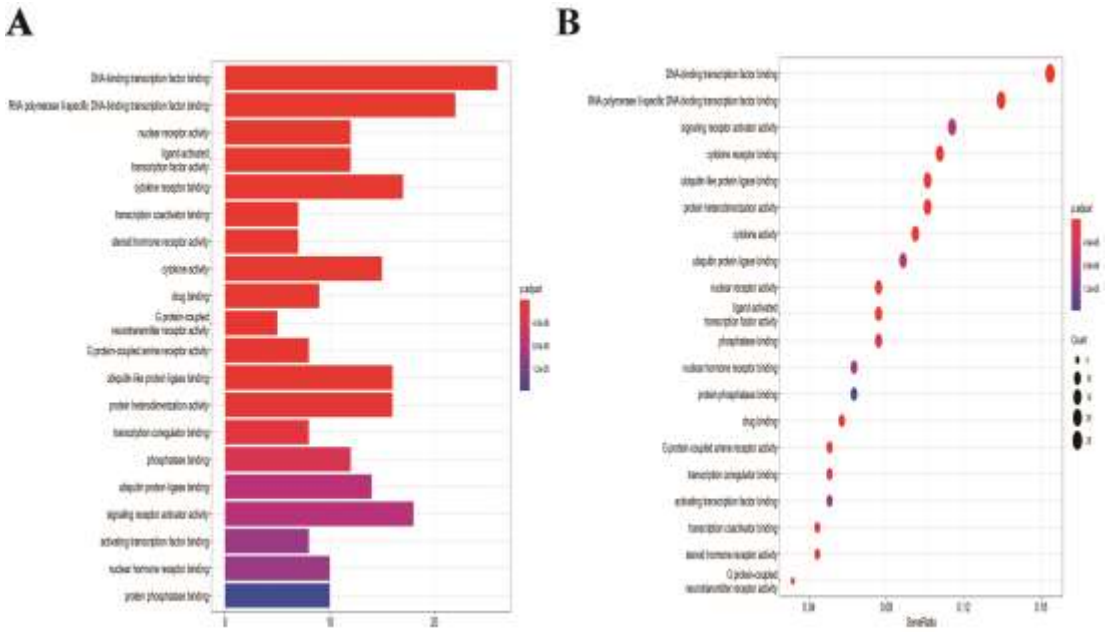

Fig 4: Histogram (A) and Bubble Diagram (B) of GO functional enrichment analysis

3.5 KEGG pathway analysis

After 158 common targets were run in R language, 171 KEGG pathways were obtained. The results of the first 20 formed a bar graph of KEGG function enrichment. P represents the significance of enrichment, and the redder the color, the higher the significance. The results show that lipid and aerosclerosis, PI3K-Akt, Human cytomegalovirus infection, Chemical carcinogenesis-receptor activation, Hepatitis B, AGE-RAGE and other signal pathways. Among them, the genes involved in lipid and atherosclerosis pathway include AKT1, BCL2, CASP9, JUN, CASP3, CASP8, MMP1, CASP7, PPARG, MAPK14, GSK3B, Bax, PRKCA, RELA, IKBKB, MAPK8, CYP1A1, ICAM1, SEL, VCAM1, PPP3CA, RXRA, BCL2L1, MM; PI3K Akt signaling pathway involves AKT1, VEGFA, BCL2, CASP9, MYC, GSK3B, CHRM1, CHRM2, PRKCA, RELA, IKBKB, INSR, RXRA, EGFR, CCND1, BCL2L1, CDKN1A, MAPK1, IL6, MDM2, ERBB2, MCL1, IL2, IL4, MET, EGF, RAF1, NOS3, COL1A1, CHUK, SPP1, IGF2, ERBB3(Fig 5).

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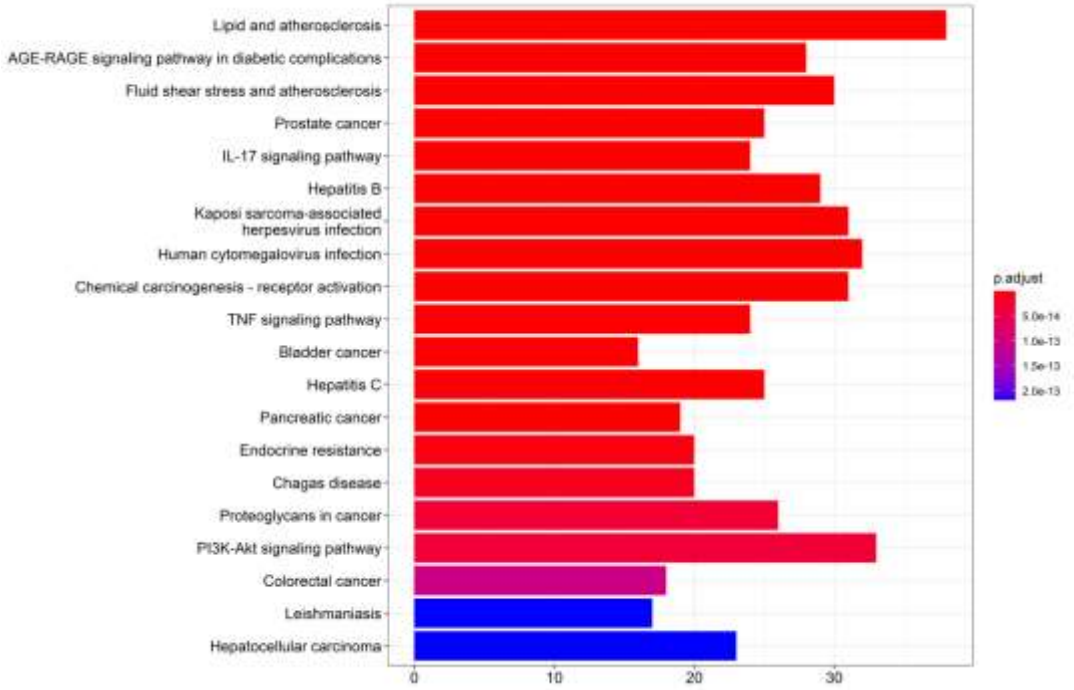

Fig 5: KEGG pathway analysis diagram

IV. DISCUSSION

According to literature reports, the chemical constituents of Honeysuckle are mainly organic acids, flavonoids, triterpene saponins, and so on. The components reported in the study are among the 23 effective compounds screened by TCMSP in this study[15]. At the same time, 1647 pharmacodynamic targets of honeysuckle were screened and 12325 gene targets related to AD were screened, including 158 targets of honeysuckle and AD. According to the screened possible target genes of honeysuckle for the remedy of AD, the gene regulation network map was established through Cytoscape and the PPI map was established through string. The gene regulation network diagram shows the characteristics of multi-component and multi-target treatment of AD. The results of PPI map showed that there was a complex interaction between the target proteins of honeysuckle. According to the number of connexins, 158 core proteins in PPI, such as PTGS1, PTGS2, GABRA1, AKT1, MMP2 and JUNVEGF2, were screened for go and KEGG enrichment pathway analysis.

Studies have shown that the main pathological product of ad-Aβ Plaques and neurofibrillary tangles (NFTs) can cause neuroinflammation and toxicity to brain tissue. The production of these neuroinflammatory cytokines is regulated by activated microglia and astrocytes. The signal pathways related to neuroinflammation in microglia include MAPK signal pathway and NF-κB, toll like receptor signaling pathway, PPAR-γ, Notch signaling pathway and JAK-STAT signaling pathway[16,17]. From the results of network analysis, it is found that the pharmacological components of honeysuckle against

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AD and the signal pathways related to neuroinflammation are MAPK and NF-κB; ERK, JNK/SAPK9 pathway and p38/MAPK pathway in MAPK pathway all play an important role in the mechanism of nerve cell injury caused by AD[18]. Therefore, functional prediction suggests that honeysuckle may inhibit A β Produce and inhibit tau protein hyperphosphorylation and A β Abnormal aggregation, weakening nerve cell apoptosis and clearing a in the brain β , Anti-inflammatory and immune activities play an ant- ad role.

Based on network pharmacology, this study connects the interaction between drugs and the body from the perspective of the overall balance of biological network, analyzes the network characteristics through the connection and relationship of nodes in biological network, further clarifies the drug action mechanism and new targets of drug action, and suggests the potential mechanism of drug treatment of diseases. Although at present the pathogenesis of AD is unclear, the honeysuckle is yet to be developed on the treatment of diseases, but with the aid of network pharmacology studies show honeysuckle can use ingredients, target and way co-ordinated intervention of AD, the occurrence and development of honeysuckle functional ingredient prevention and control of AD and targets for mechanism to provide data and theoretical support.

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REFERENCES

[1] QianYao, Hongyun Zheng, Yan Li. Research Progress on the pathogenesis of Alzheimer's disease. Occupation and Health, 2019, 35(15): 2153-2156.
[2] Nigyuan Zhang, Xijun Zheng, Lin Xu, et al. Disease progression model and research progress of Alzheimer's disease. Chinese Journal of Clinical Pharmacology and Therapeutics, 2021, 26(06): 687-694.
[3] Manxue Yin, Qingguang Wu. Bioinformatic and network pharmacological analysis of Angelica sinensis-Ligusticum chuanxiong drug pair in the treatment for Alzheimer’s disease. Chinese Traditional Patent Medicine: 1-9[2021-10-09].
[4] Chinese Pharmacopoeia Commission. Pharmacopoeia of the people's Republic of China (Volume I), 2020 Edition: China Medical Science Press, 2020.
[5] Jiao Wu, Cong Wang, Haichuan Yu. Chemical Constituents and Pharmacological Effect of Lonicerae Japonicae Flos. Chinese Journal of Experimental Traditional Medical Formulae, 2019, 25(04): 225-234.
[6] Park S, Kang S , Da S K , et al. Agrimonia pilosa Ledeb. Cinnamomum cassia Blume, and Lonicera japonica Thunb, protect against cognitive dysfunction and energy and glucose dysregulation by reducing neuroinflammation and hippocampal insulin resistance in β-amyloid-infused rats. Nutritional Neuroscience, 2017, 20(2):77-88.

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[7] Lei Luo, Bingjie Zhang, Ningning Guan, et al. In Vivo Antioxidant Activity of Honeysuckle Leaf Flavonoids in Aging Mice. Journal of Food Science, 2017, 38(19): 171-176.
[8] Jinlong Ru, Peng Li, Jinan Wang, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of Cheminformatics, 2014, 6(1).
[9] Wang Junmei, Hou Tingjun. Advances in computationally modeling human oral bioavailability. Advanced drug delivery reviews, 2015, 86.
[10] Qiwan Hu, Mudong Feng, Luhua Lai, Jianfeng Pei. Prediction of Drug-Likeness Using Deep Autoencoder Neural Networks. Frontiers in genetics, 2018, 9.
[11] Yunxia Wang, Song Zhang, Fengcheng Li, et al. Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics. Nucleic acids research, 2020, 48(D1).
[12] ZHANG J, LI B B, HUANG M Y, et al. Study on the mechanism of Shenqi Jiangtang granules in the treatment of type 2 diabetes based on network pharmacology. Chinese Journal of Chinese Herbal Medicine, 2020, 51(19): 4873-4883.
[13] Zinsmaier. Cysteine-String Protein's Neuroprotective Role. Journal of Neurogenetics, 2010, 24(3):
[14] Yan Guo, Yage Ma, Xi Zhang, et al. The lipid-lowering mechanism of walnut meal was studied based on network pharmacology. Science and Technology of Cereals, Oils and Foods,2021,29(04):144-152.
[15] Zhengwei Tan, Wei Xia, Yongliang Yu, et al. Research Progress on Chemical Constituents and Pharmacology of Honeysuckle. Journal of Anhui Agricultural Sciences, 2018, 46(09): 26-28+123.
[16] Zhang FJ, Jiang LL. Neuroinflammation in Alzheimer's disease. Neuropsychiatr Dis Treat, 2015, 2015, 11: 243−256.
[17] Wu HG, Gao Z, Luo FW, et al. Signaling pathways associated with inflammation in microglia. Curr Immunol, 2014, 34: 501−505. Wang YF, Liu S.
[18] The research progress of Alzheimer's disease and MAPKs signaling pathways. World Chin Med, 2016, 11: 1929−1931.