Mechanism of Limonoid Compounds Against Alzheimer's Disease Based on Network Pharmacology and Molecular Docking

Mao SUN, Weiqing ZHANG, Weixian YANG, Meihui DUAN, Xianji LIU*

People's Hospital of Anshun City, Anshun 561000, China

Abstract [**Objectives**] This study was conducted to explore the action mechanism of limonoids against Alzheimer's disease (AD) based on network pharmacology and molecular docking techniques. [**Methods**] Limonoid compounds were obtained through literature research (January 1942 to January 2021). Active components and potential targets of limonoids were retrieved from PubChem, TCMSP, and Swiss Target Prediction databases. AD-related targets were obtained from the GeneCards database, and intersecting targets were identified using Venny 2.1.0 to obtain the action targets of limonoids against AD. The protein-protein interaction (PPI) network was constructed using the String platform, and key targets were screened and visualized via network topology analysis with Cytoscape software. GO and KEGG pathway enrichment analyses were performed using the Metascape database, and a "drug-component-target-pathway-disease" network diagram was constructed using Cytoscape. AutoDock was empolyed for molecular docking to predict the binding properties of limonoid active components and their targets. [**Results**] A total of 60 limonoid compounds were obtained from literature research. Network pharmacology analysis showed 58 effective active components and 134 common targets between limonoids and AD. Key targets included AKT1 (serine/threonine-protein kinase 1), TNF (tumor necrosis factor), STAT3 (signal transducer and activator of transcription 3), BCL2 (B-cell lymphoma 2), and EGFR (epidermal growth factor receptor). KEGG enrichment analysis revealed key signaling pathways such as pathways in cancer, Kaposi sarcoma-associated herpesvirus infection, PI3K-Akt signaling pathway, lipid and atherosclerosis, proteoglycans in cancer, MAPK signaling pathway, and Ras signaling pathway. Molecular docking results indicated that aphanamixoid A, obacunol, cipadesin C, harpertrioate A, xylogranatin A, 11-oxocneorin G, evodulone, methyl angolensate, harrpemoid B and khivorin may be key components of limonoids against AD. [**Conclusions**] Limonoids exert anti-Alzheimer's effects through a multi-molecule, multi-target and multi-pathway mechanism.

Key words Limonoids; Alzheimer's disease; Network pharmacology; Molecular docking

DOI:10.19759/j.cnki.2164-4993.2025.04.013

Alzheimer's disease (AD; OMIM: 104300), a disease that is not unfamiliar to the elderly, is commonly known as Alzheimer's disease. It is a neurodegenerative disorder frequently occurring in old age. From the clinical characteristics, patients will experience progressive deterioration of cognitive and memory functions, as well as neuropsychiatric symptoms and behavioral disorders and other comprehensive dementia symptoms. Statistics indicate that approximately 70% of dementia cases are attributed to Alzheimer's-type dementia[1-4]. With China's continuously intensifying population aging, the prevalence of Alzheimer's disease has shown alarming growth. Recent data reveal it has become the only major disease with both rapidly increasing prevalence and a growth rate exceeding 60%. This growth rate significantly surpasses that of other diseases such as cancer, cardiovascular diseases, and AIDS[4-9]. The increasing prevalence of AD as an age-related disorder is directly correlated with the expanding elderly population base due to the aging population. AD patients not only endure tremendous personal suffering, but their care and treatment also impose substantial burdens on families and society[10]. Facing the challenges posed by AD, it is particularly urgent to delve into its pathogenesis and explore effective methods for treating or preventing the disease. Given the current shortage of investigational therapeutic drugs in the field of Alzheimer's disease (AD), accelerating the development of novel drugs and exploring innovative treatment pathways has become a critical research direction that demands breakthroughs[11-14].

Limonoids are a class of tetracyclic triterpenoid plant secondary metabolites, primarily found in plants of the Meliaceae and Rutaceae families, and relatively rare in the Simaroubaceae family. Structurally, limonoids are derived from apotirucallane or apoeuphane triterpenoid skeletons through the loss of four terminal carbon atoms from the side chain, followed by cyclization to form a 17β-furan ring. Hence, they are also referred to as tetranortriterpenoids. Limonoids exhibit a wide range of biological activities, such as anticancer, antibacterial, anti-inflammatory, and antiviral effects[15]. Tang *et al.*[16] first reported the discovery of harpertrioate A, a novel limonoid with a 7/6/6/6/5 ring system, which demonstrates significant bioactivity against AD. The study revealed that harpertrioate A targets multiple links of Aβ (extracellular β-amyloid protein produced in the brains of AD patients) production, exhibiting promising anti-AD activity. This finding provides new insights for the development of innovative AD drugs. It further confirms that limonoids can serve as active hit compounds with the potential to be developed into drugs for treating neurodegenerative diseases. However, the specific targets and

Received: March 16, 2025 Accepted: May 18, 2025

Supported by Science and Technology Fund of Guizhou health and Health Committee (gzwkj2024-240); Anshun City Science and Technology Bureau (ASKS [2024]01).

Mao SUN (1987 -), male, P. R. China, pharmacist-in-charge, PhD, devoted to research about medicinal chemistry.

* Corresponding author.

pathway-level mechanisms underlying their anti-AD effects remain unclear. Additionally, the limited availability and structural diversity of naturally isolated compounds hinder further evaluation of their anti-AD mechanisms. Therefore, in this study, network pharmacology and molecular docking were applied to systematically analyze the active components of limonoids, predict their potential anti-AD targets and mechanisms of action, and elucidate their pharmacodynamic material basis. This study will provide a theoretical foundation for further investigation into the anti-AD mechanisms of limonoid compounds.

Materials and Methods

Screening of limonoids

Relevant literature and research reports were searched using the PubMed and SciFinder databases. The key word "limonoids" was used for a subject search (January 1942 to January 2021). From the retrieved literature, limonoid compounds with structures similar or identical to the limonoid skeleton of harpertrioate A, while also exhibiting structural diversity, were selected. The obtained compounds were verified using the PubChem database[17] (https://pubchem.ncbi.nlm.nih.gov). For compounds not included in the database, their structures were drawn using ChemDraw, and their Canonical SMILES codes were copied or their 2D structures were downloaded. The 2D structures of the compounds were saved in SDF format files.

Prediction of limonoid active component targets

The Canonical SMILES codes or SDF-formatted 2D structural files of the limonoid active compounds were uploaded to the Swiss Target Prediction database[18] (http://swisstargetprediction.ch/). The species was set to *Homo sapiens*, and the "Predict targets" function was clicked to generate compound target information. The results were exported and saved in CSV format. The target data of chemical components were merged using Excel, and targets with a probability > 0 in the prediction results were selected. De-duplicaion was performed to obtain the bioactive component targets. For compounds without predicted targets, their InChIKeys were uploaded to the TCMSP database[19] (https://old.tcmsp-e.com/tcmsp.php). All targets listed under "Related targets" were copied into an Excel datasheet. The data were processed by uploading the "Target name" column to the UniProt database[20] (https://www.uniprot.org/) and searching for corresponding gene names. The retrieved data were downloaded, and the collected targets were standardized to official names before removing duplicates. The results from both prediction methods were merged.

Collection of AD-related targets and acquisition of common targets

After accessing the GeneCards database[21], targets related to Alzheimer's disease (AD) were screened and collected using "Alzheimer's disease" as the search term. The retrieved results were downloaded and opened in Excel, where AD-related targets with a Relevance score ≥ 20 were filtered. The limonoid bioactive component targets and AD-related targets were intersected to obtain common targets using the Venny 2.1.0 online tool (http://www.liuxiaoyuyuan.cn/).

Construction of protein-protein interaction (PPI) network for limonoid and AD targes

The obtained common targets were imported into the STRING online platform[22] (https://cn.string-db.org/). The "Multiple proteins" option was selected, while setting the speices to *H. sapiens* and the minimum required interaction score ≥ 0.4. The protein interaction results in TSV format were downloaded and imported into Cytoscape 3.10.0 software[23] for visualization and analysis. The core targets were screened using the Network Analyzer plugin, with degree, betweenness and closeness as evaluation indicators. The top 30 intersecting targets ranked comprehensively on the basis of exceeding the median values of the three parameters were selected as potential key targets.

GO enrichment and KEGG pathway analysis

The TSV-formatted files from STRING database were processed by merging node1 and node2 data and removing duplicate values. Next, the processed data were imported into Metascape database[24] (http://metascape.org/gp/index.html#/main/step1) for GO functional enrichment and KEGG pathway analysis. The analysis was initiated by clicking Submit, followed by selecting *H. sapiens* (166) as the target species. The Custom Analysis option was selected, followed by clicking Enrichment, and GO Biological Processes, GO Cellular Components, GO Molecular Functions and KEGG Pathways were selected respectively for analysis. The resulting data were downloaded and subsequently visualized as GO and KEGG diagrams using the online plotting tools provided by the Bioinformatics online platform (http://www.bioinformatics.com.cn/).

Construction of the "limonoid-active component-AD-target-pathway" network

After preparing the network and type files for "limonoid-components-AD-target-pathway", Cytoscape 3.10.0 software was used for visualization to construct the "limonoid-component-AD-target-pathway" network. The Network Analyzer plugin was employed to screen core active components, which were then exported to an Excel file. Using degree, betweenness and closeness as evaluation indicators, the components were comprehensively ranked, and the top 10 active components were selected as key candidates for subsequent molecular docking.

Molecular docking simulation validation of key components and targets

Molecular docking was performed between the key components and key targets identified from the "protein-protein interaction (PPI) network" and the "limonoid-active component-AD-target-pathway network". The 2D or 3D structures of the key components were downloaded from the PubChem or TCMSP databases. The structures obtained from PubChem were saved in SDF format and converted to mol2 format using Chem3D 20.0 software. Autoduck software was then employed to add hydrogen atoms, and the

files in pdbqt format were exported after steps of setting as ligands, detecting rotatable bonds and setting rotatable bonds. The PDB structures of key targets were downloaded from the PDB database. Pymol software was used to remove water molecules, ligands, and solvent molecules from the protein structures. Autoduck software was then employed to add hydrogen atoms to the processed structures, which were then set as receptors and exported in pdbqt format. Finally, molecular docking was performed using Autoduck software, and the docking results were imported into Pymol software for visualization, generating molecular docking diagrams.

Results and Analysis

Screening of limonoid compounds

Literature search was conducted using PubMed and SciFinder databases to identify limonoid compounds with skeletons similar or identical to harpertrioate A while maintaining structural diversity. A total of 60 limonoid compounds were obtained[25-27]. The basic information of these compounds is presented in Table 1.

Table 1 Screening results of limonoids

No.	Compound	Molecular formula	Molecular weight
LM1	7-Benzoyl-17-hydroxynimbocinol	$C_{33}H_{36}O_6$	528.65
LM2	Walsurin	$C_{26}H_{32}O_5$	424.22
LM3	17-Epiazadiradione	$C_{28}H_{34}O_5$	450.58
LM4	Cedrelone	$C_{26}H_{30}O_5$	422.52
LM5	Hirtin	$C_{32}H_{36}O_{11}$	596.63
LM6	1,2-Dihydrocedrelone	$C_{26}H_{32}O_5$	424.54
LM7	Havanensin	$C_{26}H_{38}O_5$	430.59
LM8	Deoxykhayanthone	$C_{32}H_{42}O_8$	554.68
LM9	Nilotin	$C_{40}H_{52}O_{14}$	756.84
LM10	Trichilin A	$C_{35}H_{46}O_{13}$	674.74
LM11	Aphanastatin	$C_{35}H_{46}O_{13}$	674.74
LM12	Amoorastatone	$C_{28}H_{36}O_9$	516.59
LM13	Vilasinin	$C_{26}H_{36}O_5$	428.57
LM14	Trichilinin	$C_{30}H_{40}O_8$	528.64
LM15	Malleastrone C	$C_{32}H_{40}O_{11}$	600.66
LM16	Meldenin	$C_{28}H_{38}O_5$	454.61
LM17	Isomeldenin	$C_{28}H_{38}O_5$	454.61
LM18	1,2-Dihydroazadirone	$C_{30}H_{40}O_6$	496.64
LM19*	Evodulone	$C_{28}H_{34}O_7$	482.57
LM20	Surenone	$C_{26}H_{32}O_6$	440.54
LM21	Rubralin C	$C_{35}H_{46}O_9$	610.74
LM22	Quivisianthone	$C_{33}H_{42}O_9$	582.69
LM23	Turrapubesin C	$C_{31}H_{40}O_{10}$	572.65
LM24	Salannin	$C_{34}H_{44}O_9$	596.72
LM25	Ohchinal	$C_{35}H_{40}O_8$	588.70
LM26	Nimbin	$C_{30}H_{36}O_9$	540.61
LM27	Ohchinolal	$C_{34}H_{44}O_{10}$	612.72
LM28	Gedunin	$C_{28}H_{34}O_7$	482.57
LM29*	Khivorin	$C_{32}H_{42}O_{10}$	586.68
LM30	Azadirinin	$C_{39}H_{46}O_{10}$	674.79
LM31	Nimolicinol	$C_{28}H_{34}O_7$	482.57
LM32	Piscidofuran	$C_{33}H_{42}O_{10}$	598.69
LM33	Surenolactone	$C_{26}H_{32}O_6$	440.54
LM34*	Obacunol	$C_{26}H_{32}O_7$	456.54
LM35	Dysoxylone	$C_{31}H_{38}O_{10}$	570.64
LM36*	11-Oxocneorin G	$C_{30}H_{36}O_{11}$	572.61
LM37*	Methyl angolensate	$C_{27}H_{34}O_7$	470.56
LM38	Cipadesin D	$C_{33}H_{40}O_{13}$	644.67
LM39	Methyl ivorensate	$C_{27}H_{34}O_8$	486.56
LM40	Mexicanolide	$C_{27}H_{32}O_7$	468.55
LM41	Carapin	$C_{27}H_{32}O_7$	468.55
LM42*	Xylogranatin A	$C_{34}H_{42}O_{12}$	642.70
LM43	Grandifotane A	$C_{29}H_{36}O_{11}$	560.60
LM44	Moluccensin I	$C_{30}H_{36}O_{10}$	556.61
LM45	Tabulalide E	$C_{35}H_{44}O_{18}$	752.72
LM46*	Cipadesin C	$C_{31}H_{38}O_{11}$	586.63
LM47	Cipadonoid F	$C_{31}H_{40}O_{11}$	588.65
LM48	Walsuronoid B	$C_{26}H_{30}O_6$	438.52
LM49	Nimbinene	$C_{28}H_{34}O_7$	482.57
LM50	Ceramicine A	$C_{26}H_{32}O_6$	440.54
LM51	Xylogranatin F	$C_{26}H_{27N}O_6$	449.50
LM52	Toonaciliatin G	$C_{25}H_{32}O_9$	476.52
LM53	Trijugin D	$C_{28}H_{32}O_{11}$	544.55
LM54	Cipadonoid D	$C_{31}H_{38}O_{10}$	570.64
LM55	Perforanoid A	$C_{25}H_{28}O_6$	424.49
LM56*	Aphanamixoid A	$C_{29}H_{36}O_7$	496.60
LM57	Haperforin G	$C_{25}H_{28}O_6$	424.49
LM58*	Harrpemoid B	$C_{25}H_{30}O_7$	442.51
LM59	Cipadonoid A	$C_{24}H_{34}O_6$	418.53
LM60*	Harpertrioate A	$C_{27}H_{34}O_9$	502.22

(Continued) (Table 1)

Those marked with * are the key components.

Screening of limonoid active components and target prediction

Target prediction for the 60 compounds was performed using TCMSP and Swiss Target Prediction databases. Compounds LM8 (deoxykhayanthone) and LM44 (moluccensin I) showed no detectable active substances or predicted targets in the results, so 58 active components were identified. After removing duplicate targets, a total of 670 potential targets were obtained.

Screening of AD targets and identification of common targets

A total of 15 512 AD-related targets were obtained from the Genecards database. Targets with a Relevance score ≥20 were selected as potential therapeutic targets, yielding 1 117 relevant targets. The 670 potential targets of limonoid active components were intersected with the 1 117 AD-related targets from the Genecards database using Venny 2.1.0. The intersection analysis identified 134 common targets, as shown in the Venn diagram (Fig. 1).

Construction of protein-protein interaction network for the anti-AD action targets of limonoids

A PPI network of common targets of limonoids against AD was constructed using the String online database, and the results

were visualized and analyzed using Cytoscape 3.10.0, as shown in Fig. 2. Among the 134 common targets, all 134 were interconnected, forming 2 046 interaction relationships. The color and size of the nodes were positively correlated with their degree values. Based on a comprehensive ranking of degree, betweenness and closeness values, the top 30 common targets were identified through data sorting and screening in Excel, as shown in Table 2. These targets were predicted to be the key targets of limonoids.

Table 2 Key targets and topological characteristics of limonoids

Name	Degree	Closeness	Betweenness
AKT1	98	0.791 7	0.071 1
TNF	95	0.777 8	0.087 2
STAT3	83	0.722 8	0.027 1
BCL2	83	0.718 9	0.028 0
EGFR	82	0.718 9	0.040 8
CASP3	81	0.715 1	0.025 6
SRC	78	0.707 4	0.035 2
JUN	77	0.700 0	0.033 0
PPARG	75	0.689 1	0.045 8
HIF1A	73	0.685 6	0.018 7
GSK3B	71	0.675 1	0.033 1
MAPK3	69	0.671 7	0.024 8
MTOR	65	0.655 2	0.010 0
APP	64	0.655 2	0.040 3
MMP9	64	0.655 2	0.009 5
PTGS2	62	0.648 8	0.011 0
ERBB2	57	0.630 3	0.006 3
CCND1	57	0.627 4	0.006 5
JAK2	54	0.621 5	0.008 2
MMP2	52	0.618 6	0.005 1
RHOA	51	0.612 9	0.004 0
IGF1R	51	0.610 1	0.004 9
PIK3CA	51	0.596 4	0.003 5
ICAM1	50	0.612 9	0.006 1
KDR	49	0.607 3	0.012 3
MAPK1	49	0.607 3	0.007 6
PTPN11	47	0.599 1	0.004 0
MAPK14	45	0.596 4	0.007 2
CASP8	44	0.588 5	0.003 0
CASP1	41	0.583 3	0.007 4

GO functional annotation and KEGG pathway enrichment analysis

To elucidate the biological processes involved in the anti-AD effects of limonoids, the TSV-format files from the STRING database were processed by merging node1 and node2 data and removing duplicate values, resulting in 134 gene targets. GO and KEGG analyses were then performed using the Metascape database. Screening was performed with a P Value Cutoff = 0.01, and analysis was conducted from the aspects of biological processes (BP), cellular components (CC), and molecular functions (MF). A total of 1 792 BP items, 120 CC items and 214 MF items were obtained. After sorting by LogP value in ascending order, the top 10 items in each category were analyzed, and the results are shown in Fig. 3.

GO analysis revealed that in terms of biological processes (BP), the main functions included cellular response to nitrogen compounds, phosphorylation, positive regulation of phosphorus metabolic processes, positive regulation of phosphate metabolic processes, protein phosphorylation, cell surface receptor protein tyrosine kinase signaling pathway, positive regulation of locomotion, positive regulation of phosphorylation, enzyme-linked receptor protein signaling pathway, and positive regulation of cell motility. For cellular components (CC), the targets were primarily associated with membrane rafts, membrane microdomains, receptor complexes, dendrites, dendritic trees, caveolae, cell body, axon, neuronal cell body, and plasma membrane rafts. In terms of molecular functions (MF), the targets were primarily associated with protein kinase activity, phosphotransferase activity, kinase activity, histone H2AX kinase activity, histone H3 kinase activity, histone kinase activity, histone H3Y41 kinase activity, histone H2AXY142 kinase activity, protein tyrosine kinase activity, and histone modification activity.

KEGG pathway enrichment analysis identified a total of 185 pathways. A bubble chart of the top 20 KEGG pathways ranked by LogP value was generated, as shown in Fig. 4. The results indicated that the active components of limonoids primarily exerted their anti-Alzheimer's disease effects through pathways such as pathways in cancer, Kaposi sarcoma-associated herpesvirus infection, PI3K-Akt signaling pathway, lipid and atherosclerosis, proteoglycans in cancer, MAPK signaling pathway, Ras signaling pathway, hepatitis B, Rap1 signaling pathway, human cytomegalovirus infection, prostate cancer, neurotrophin signaling pathway, endocrine resistance, central carbon metabolism in cancer, HIF-1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, colorectal cancer, AGE-RAGE signaling pathway in diabetic complications, PD-L1 expression and PD-1 checkpoint pathway in cancer, and prolactin signaling pathway.

Table 3 Key active components and their topological features

Active component	Degree	Betweennes	Closeness
LM56	45	0.030 374 097	0.449 367 089
LM34	42	0.032 474 261	0.445 606 695
LM46	42	0.031 285 153	0.445 606 695
LM60	41	0.017 319 849	0.443 750 000
LM42	40	0.029 546 638	0.441 908 714
LM36	40	0.024 195 346	0.441 908 714
LM19	40	0.022 322 860	0.441 908 714
LM37	40	0.021 853 552	0.441 908 714
LM58	39	0.016 368 073	0.440 082 645
LM29	39	0.014 477 917	0.438 271 605

Construction of the limonoid-active component-target-disease-pathway network

The 20 KEGG pathways from the above enrichment analysis, 134 interacting potential targets and 58 active components were jointly analyzed. Using Cytoscape 3.10.0 software, a "drug-component-target-disease-pathway network diagram" was constructed, as shown in Fig. 5. Topological analysis was performed using the

"Network Analyzer" function, with degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) as evaluation indicators. The top 10 active components ranked comprehensively were identified as key active ingredients: LM56 (aphanamixoid A), LM34 (obacunol), LM46 (cipadesin C), LM60 (harpertrioate A), LM42 (xylogranatin A), LM36 (11-oxocneorin G), LM19 (evodulone), LM37 (methyl angolensate), LM58 (harrpemoid B), and LM29 (khivorin).

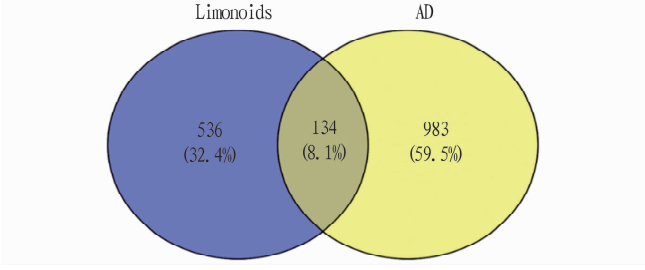


Fig. 1 Common targets of limonoids and Alzheimer's disease

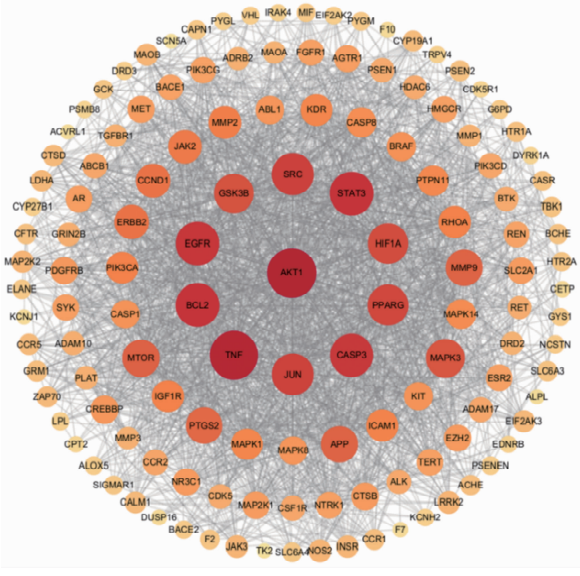


Fig. 2 PPI network of limonoids-AD common targets

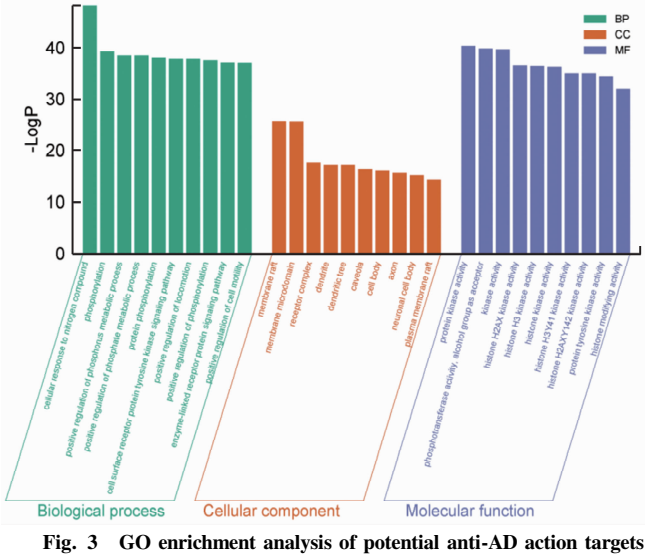


Fig. 3 GO enrichment analysis of potential anti-AD action targets

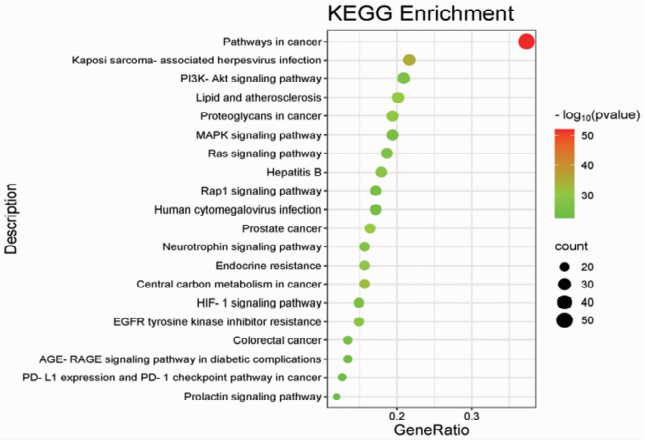
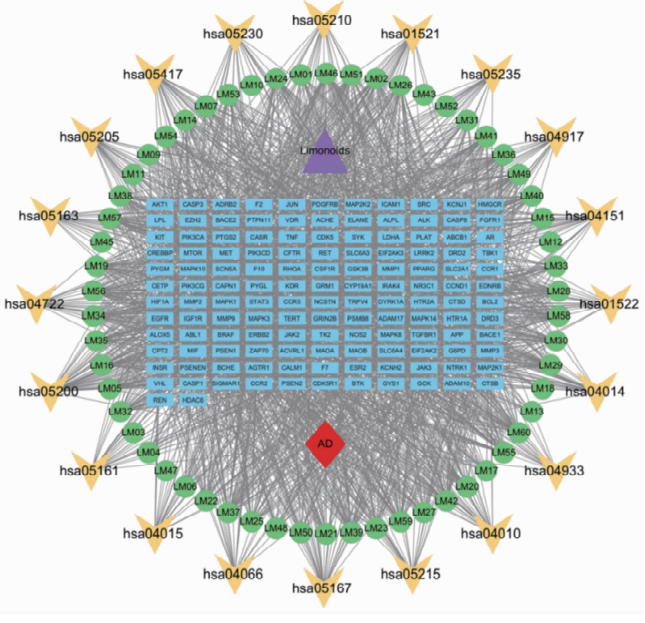


Fig. 4 KEGG pathway enrichment analysis of potential limonoid targets against Alzheimer's disease



Blue: target; Yellow: pathway; Red: disease; Purple: drug; Green: active component.

Fig. 5 Drug-component-disease-target-pathway network

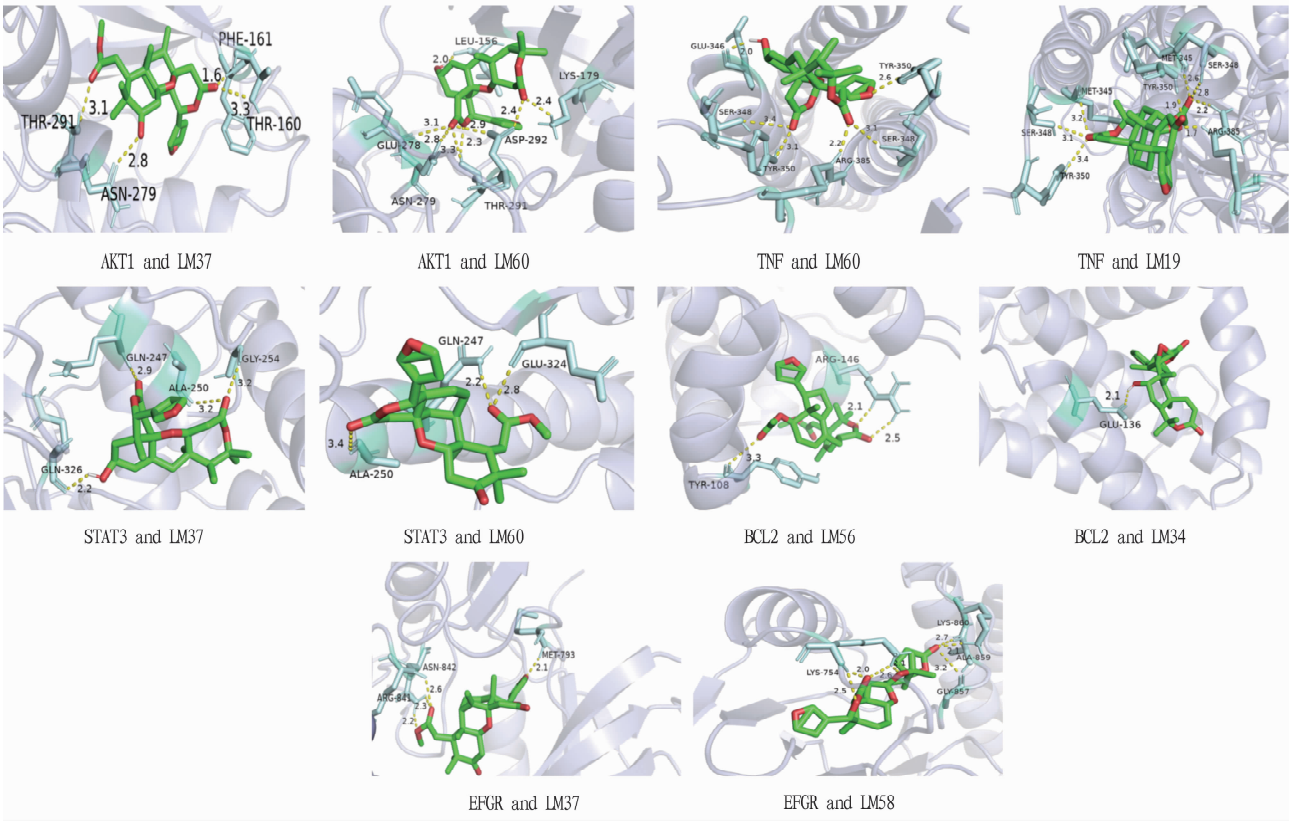
Molecular docking validation of key active components and targets

The top 10 key active components (aphanamixoid A, obacunol, cipadesin C, harpertrioate A, xylogranatin A, 11-oxocneorin G, evodulone, methyl angolensate, harrpemoid B, khivorin) were selected based on a comprehensive ranking of degree value, betweenness centrality, and closeness centrality in the "drug-component-target-disease-pathway" network. Molecular docking was performed with the top 5 key targets from the PPI network: AKT1 (serine/threonine-protein kinase 1), TNF (tumor necrosis factor), STAT3 (signal transducer and transcriptional activator 3), BCL2 (B-cell lymphoma 2 protein), and EGFR (epidermal growth factor receptor). The binding energy results are shown in Table 4. The binding affinity between proteins and ligands was evaluated based on docking binding energy (a larger absolute docking score indicates stronger binding affinity). Generally, a binding

energy ≤ −4.25 Kcal/mol suggests certain binding activity between the active component and the target; a binding energy ≤ −5.0 kcal/mol indicates good binding activity between them; and a binding energy ≤ −7.0 kcal/mol signifies strong binding activity[28]. The binding energies of all molecular docking results were below −4.25 Kcal/mol, indicating that all key molecules exhibited binding activity. Among them, 35 pairs had binding energies below −7.0 Kcal/mol, suggesting strong binding affinity between these active components and their targets. The key components and key targets could spontaneously bind to exert anti-AD effects. The optimal docking results were visualized using Pymol software, as shown in Fig. 6.

Table 4 Molecular docking binding energies of key components and key targets Kcal/mol

No.	Compound	AKT1 (4gv1)	TNF (1ca9)	STAT3 (6njs)	BCL2 (6gl8)	EGFR (5hg8)
LM56	Aphanamixoid A	−8.53	−7.24	−5.78	−9.09	−8.43
LM34	Obacunol	−8.59	−7.99	−6.47	−8.32	−8.46
LM46	Cipadesin C	−7.34	−5.59	−5.10	−6.87	−7.96
LM60	Harpertrioate A	−8.80	−8.59	−7.21	−7.91	−7.61
LM42	Xylogranatin A	−7.96	−4.59	−4.55	−6.85	−7.40
LM36	11-Oxocneorin G	−8.48	−7.53	−5.98	−7.99	−8.13
LM19	Evodulone	−8.65	−8.47	−6.07	−7.86	−8.37
LM37	Methyl angolensate	−8.80	−7.61	−6.92	−7.79	−9.07
LM58	Harrpemoid B	−8.75	−8.24	−6.68	−7.80	−9.40
LM29	Khivorin	−7.92	−6.33	−5.24	−7.50	−6.92



AKT1 and LM37 AKT1 and LM60 TNF and LM60 TNF and LM19

STAT3 and LM37 STAT3 and LM60 BCL2 and LM56 BCL2 and LM34

EFGR and LM37 EFGR and LM58

Fig. 6 Molecular docking results of key components and targets

Conclusions and Discussion

In this study, limonoids were obtained by searching related research literature of limonoids, and based on the structure of harpertrioate A, which exhibits anti-AD activity, limonoids with similar or identical skeleton and structural diversity were screened, resulting in 58 active compounds. Additionally, 670 predicted targets of these active components were obtained, and 134 common targets were identified through intersection with disease-related targets. Through PPI network analysis, the top five key targets with the highest degree values were identified as AKT1 (serine/threonine-protein kinase 1), TNF (tumor necrosis factor), STAT3 (signal transducer and transcriptional activator 3), BCL2 (B-cell lymphoma 2 protein), and EGFR (epidermal growth factor receptor). They showed obvious correlation with other targets, and may

be the key targets for the anti-AD effects of limonoids. The "drug-component-disease-target-pathway" network visually demonstrates that limonoid active components exert anti-AD effects through multiple targets and pathways. Molecular docking results of key active components with core targets suggest that aphanamixoid A, obacunol, cipadesin C, harpertrioate A, xylogranatin A, 11-oxocneorin G, evodulone, methyl angolensate, harrpemoid B, and khivorin all exhibit strong binding affinity with key targets, indicating they may serve as the primary active components responsible for the potential anti-AD effects of limonoid compounds.

Currently, the widely recognized molecular pathological features specific to AD include extracellular β-amyloid (Aβ) plaque deposition in the brain and intraneuronal neurofibrillary tangles formed by hyperphosphorylated microtubule-associated protein (Tau hyperphosphorylation)[29]. The mechanisms primarily focus on Aβ and MAPT/tau pathways. Studies have shown that AKT1 participates in regulating tau protein phosphorylation and Aβ production through the PI3K/AKT pathway. On one hand, Aβ oligomers inhibit AKT1 activity, leading to excessive activation of GSK3β, which promotes abnormal tau phosphorylation and the formation of neurofibrillary tangles[30]. On the other hand, reduced AKT1 activity decreases membrane localization of TACE (TNF-α converting enzyme), resulting in abnormal APP cleavage and increased Aβ42 generation, exacerbating amyloid plaque deposition[31]. In recent years, AKT1-targeted AD treatment strategies have demonstrated potential. For example, CRISPR-Cas9-mediated AKT1 knockout significantly reduced amyloid plaques and improved cognitive function in AD mouse models[32]. Meanwhile, the Chinese herbal medicine Wuling Capsule achieved multi-target neuroprotection by activating the PI3K/AKT pathway and regulating AKT1 and downstream inflammatory factors[33]. These studies collectively indicate that AKT1 serves not only as a central node in AD pathological mechanisms but also as a crucial target for drug development. TNF-α plays a pivotal role in promoting AD pathogenesis by activating the NF-κB pathway to drive neuroinflammation, accelerate Aβ deposition and tau phosphorylation, and induce neuronal apoptosis[34-35]. Studies have revealed STAT3's involvement in AD progression, as Aβ oligomers activate STAT3 to exacerbate neuroinflammation through upregulation of pro-inflammatory factors like IL-6, while simultaneously suppressing expression of neuron survival-related genes[36]. In AD patients, the expression of BCL2 in the brain is downregulated, leading to increased mitochondrial membrane permeability, cytochrome C release, and ultimately triggering neuronal apoptosis[37]. EGFR can activate the MAPK/ERK and PI3K/Akt signaling pathways, inducing the autophagy mechanism in neuronal cells to clear damaged mitochondria in the brain, thereby mitigating the progression of Alzheimer's disease caused by mitochondrial dysfunction and cumulative damage[38-39]. The above research indicates that key targets such as AKT1, TNF, STAT3, BCL2, and EGFR are all involved in the pathogenesis of AD. Regulating these targets can play a crucial role in the treatment or prevention of AD.

GO analysis revealed that limonoids could modulate the pathological progression of AD by influencing biological processes such as cellular response to nitrogen compounds, phosphorylation, and positive regulation of phosphorus metabolic processes. Abnormal accumulation of nitrogen compounds (such as glutamate and nitric oxide) exacerbates neuronal damage through excitotoxicity and oxidative stress[40-41]. Phosphorylation imbalance leads to hyperphosphorylation of tau protein and the formation of neurofibrillary tangles, and promotes Aβ production via the GSK-3β pathway[42]. Disordered phosphorus metabolism disrupts mitochondrial energy metabolism, triggers calcium dyshomeostasis, and impairs autophagy function, which is one of the core mechanisms of neurodegenerative pathology in AD[43-46]. KEGG pathway enrichment analysis revealed that the anti-AD effects of limonoid active components were primarily associated with pathways such as pathways in cancer, Kaposi sarcoma-associated herpesvirus infection (KSHV infection), PI3K-Akt signaling pathway, lipid and atherosclerosis, proteoglycans in cancer, MAPK signaling pathway, Ras signaling pathway, hepatitis B, Rap1 signaling pathway, and human cytomegalovirus infection. These pathways are closely linked to AD treatment. For example, in cancer-related pathways, AD and cancer share core mechanisms such as inflammation, oxidative stress, and metabolic dysregulation. Abnormal activation of pathways like mTOR and MAPK can promote Aβ deposition and tau protein phosphorylation, leading to formation of neurofibrillary tangles[47]. Regarding the KSHV infection pathway, KSHV downregulates the anti-inflammatory molecule lipoxin A4 and activates the NF-κB and Akt pathways, exacerbating neuroinflammation, which may contribute to the chronic inflammatory pathology of AD[48-49]. In the PI3K-Akt signaling pathway, Akt activity is inhibited by sulfhydrylation in AD, leading to GSK-3β overactivation, which promotes tau phosphorylation and synaptic dysfunction[50]. This pathway also contributes to AD-related energy metabolism dysregulation by modulating autophagy and mitochondrial function[51].

In summary, in this study, network pharmacology and molecular docking were applied to explore the material basis and mechanism of limonoids against Alzheimer's disease. The results demonstrate that the anti-AD effects of limonoids exhibit multi-component, multi-target, multi-pathway, and multi-mechanism characteristics. These findings also suggest the potential therapeutic value of limonoids in AD treatment, providing a theoretical foundation and scientific basis for further in-depth research.

References

[1] BURNS A, ILIFFE S. Alzheimer's disease[J]. BMJ, 2009, 338: b158.
[2] HUANG Y, MUCKE L. Alzheimer mechanisms and therapeutic strategies [J]. Cell, 2012, 148: 1204-1222.
[3] BÄCKMAN L, JONES S, BERGER AK, *et al.* Multiple cognitive deficits during the transition to Alzheimer's disease[J]. J Intern Med, 2004, 256: 195-204.
[4] Alzheimer's Association. 2014 Alzheimer's disease facts and figures[J]. Alzheimers Dement, 2014,10(2): e47-92.
[5] PRINCE M, WIMO A, GUERCHET M, *et al.* Alzheimer's Disease International; London: 2015[J]. World alzheimer report, 2015.
[6] JIA J,WANG F, WEI C, *et al.* The prevalence of dementia in urban and rural areas of China[J]. Alzheimers Dement, 2014, 10: 1-9.
[7] CHAN KY, WANG W, WU JJ, *et al.* Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990-2010: A systematic review and analysis[J]. Lancet, 2013, 381: 2016-2023.

[8] DOODY RS, RAMAN R, FARLOW M, *et al.* A phase 3 trial of semagacestat for treatment of Alzheimer's disease[J]. N Engl J Med, 2013, 369: 341 – 350.
[9] SALLOWAY S, SPERLING R, FOX NC, *et al.* Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease[J]. N Engl J Med, 2014, 370: 322 – 333.
[10] DOODY RS, THOMAS RG, FARLOW M, *et al.* Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease[J]. N Engl J Med., 2014, 370(4): 311 – 321.
[11] ATRI A, FRÖLICH L, BALLARD C, *et al.* Effect of idalopirdine as adjunct to cholinesterase inhibitors on change in cognition in patients with Alzheimer disease: Three randomized clinical trials[J]. JAMA, 2018, 319(2): 130.
[12] BRIGGS R, KENNELLY SP, O'NEILL D. Drug treatments in Alzheimer's disease[J]. Clin Med (Lond), 2016, 16(3): 247.
[13] CUMMINGS JL, MORSTORF T, ZHONG K. Alzheimer's disease drug-development pipeline: Few candidates, frequent failures[J]. Alzheimers Res Ther, 2014, 6(4): 37.
[14] WELLER J, BUDSON A. Current understanding of Alzheimer's disease diagnosis and treatment[J]. F1000Research, 2018, 7: F1000 Faculty Rev – 1161.
[15] ZHANG WQ, JI HX, WU Q, *et al.* Overview of researches on limonoids and their biological activities[J]. Anhui Agricultural Science Bulletin, 2024, 30(1): 21 – 26. (in Chinese).
[16] TANG XH, LUO RC, YE MS, *et al.* Harpertrioate A, an A,B,D-seco-limonoid with promising biological activity against Alzheimer's disease from twigs of *Harrisonia perforata* (Blanco) Merr[J]. Org Lett., 2021, 23(2): 262 – 267.
[17] KIM S, CHEN J, CHENG T, *et al.* PubChem in 2021: New data content and improved web interfaces[J]. Nucleic Acids Res., 2021, 49 (D1): D1388 – D1395.
[18] GFELLER D, MICHIELIN O, ZOETE V. Shaping the interaction landscape of bioactive molecules[J]. Bioinformatics, 2013, 29(23): 3073 – 3079.
[19] RU J, LI P, WANG J, *et al.* TCMSP: A database of systems pharmacology for drug discovery from herbal medicines[J]. J Cheminform., 2014, 6: 13.
[20] UniProt Consortium. UniProt: The universal protein knowledgebase in 2023[J]. Nucleic Acids Res., 2023, 51(D1): D523 – D531.
[21] RAPPAPORT N, TWIK M, PLASCHKES I, *et al.* MalaCards: An amalgamated human disease compendium with diverse clinical and genetic annotation and structured search[J]. Nucleic Acids Res., 2017, 45 (D1): D877 – D887.
[22] SZKLARCZYK D, KIRSCH R, KOUTROULI M, *et al.* The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest[J]. Nucleic Acids Res., 2023, 51(D1): D638 – D646.
[23] KOHL M, WIESE S, WARSCHEID B. Cytoscape: Software for visualization and analysis of biological networks[J]. Methods in Molec Bio, 2011, 696: 291 – 303.
[24] ZHOU Y, ZHOU B, PACHE L, *et al.* Metascape provides a biologist-oriented resource for the analysis of systems-level datasets[J]. Nat Commun., 2019, 10(1): 1523.
[25] LI FX, WANG JJ. Research status of limonoids[J]. International Journal of Traditional Chinese Medicine, 2000(3): 131 – 135, 147. (in Chinese).
[26] TAN QG, LUO XD. Meliaceous limonoids: Chemistry and biological activities[J]. Chem Rev., 2011, 111(11): 7437 – 7522.
[27] LYU C, YAN X, TU Q, *et al.* Isolation and asymmetric total synthesis of perforanoid A[J]. Angew Chem Int Ed Engl., 2016, 55(26): 7539 – 7543.
[28] GAILLARD T. Evaluation of AutoDock and AutoDock Vina on the CASF – 2013 benchmark[J]. Journal of Chemical Information and Modeling, 2018, 58(8): 1697 – 1706.
[29] EHRET MJ, CHAMBERLIN KW. Current practices in the treatment of Alzheimer disease: Where is the evidence after the phase III trials[J]. Clin Ther, 2015, 37: 1604 – 1616, .
[30] AHMAD F, SINGH K, DAS D, *et al.* Reactive oxygen species-mediated loss of synaptic akt1 signaling leads to deficient activity-dependent protein translation early in Alzheimer's disease[J]. Antioxid Redox Signal, 2017, 27(16): 1269 – 1280.
[31] YANG S, DU Y, ZHAO X, *et al.* Reducing PDK1/Akt activity: An effective therapeutic target in the treatment of Alzheimer's disease[J]. Cells, 2022, 11(11): 1735.
[32] Tongfang Co., Ltd., Tsinghua University. A gene therapy targeting PDK1/AKT in cerebral cortex neurons for treating Alzheimer's disease: China, CN202410123456.7[P]. 2024 – 03 – 15 (2024 – 08 – 16). (in Chinese).
[33] MA T. Network pharmacology-based exploration of the anti-Alzheimer's disease mechanism of Wuling Capsule via PI3K/AKT signaling pathway[J]. Future Integrative Medicine, 1(1): 1 – 12.
[34] XING GH, LI XY, LIN CR, *et al.* Expression levels and clinical application value of TNF-α, M-CSF and miR-181a in serum of elderly AD patients[J]. Journal of Hebei Medical University, 2023, 44 (9): 1006 – 1010. (in Chinese).
[35] LEMPRIÈRE S. Genome-wide association study identifies new risk loci for Alzheimer disease[J]. Nature Reviews Neurology, 2021, 17(11): 659.
[36] PENG X, WANG Z. NSTs improves cognitive function of Alzheimer's disease model mice by inhibiting STAT3-regulated neuroinflammation[J]. Journal of Nanhua University: Medical Edition, 2024, 52(3): 421 – 426. (in Chinese).
[37] XING GH, LI XY, LIN CR, *et al.* Expression of Bcl-2 and Bax in hippocampus of senile dementia model rats and the intervention effect of Qifuyin[J]. Chinese Journal of Gerontology, 2010, 30(10): 1389 – 1391.
[38] CHEN W, CHEN HR, XIE JM. Exploration of the molecular mechanism of Codonopsis anti-Alzheimer's disease based on net-work pharmacology and molecular docking[J]. Lishizhen Medicine and Materia Medica Research, 2024, 35(14): 3295 – 3304. (in Chinese).
[39] MAO YP, SONG YM, PAN SW, *et al.* Effect of Codonopsis Radix and Polygonati Rhizoma on the regulation of the IRS1/PI3K/AKT signaling pathway in type 2 diabetic mice[J]. Front Endocrinol (Lausanne), 2022, 13: 1068555.
[40] HYND MR, SCOTT HL, DODD PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease[J]. Neurochemistry International, 2024, 45(5): 583 – 595.
[41] WEIDLING I, SWERDLOW RH. Mitochondrial dysfunction and stress responses in Alzheimer's disease[J]. Biology, 2019, 8(2): 39.
[42] DENG J, HABIB A, OBREGON DF, *et al.* Soluble amyloid precursor protein alpha inhibits tau phosphorylation through modulation of GSK3β signaling pathway[J]. J Neurochem., 2015, 135(3): 630 – 637.
[43] RIJPMA A, VAN DER GRAAF M, MEULENBROEK O, *et al.* Altered brain high-energy phosphate metabolism in mild Alzheimer's disease: A 3-dimensional 31P MR spectroscopic imaging study[J]. NeuroImage: Clinical, 2018, 18: 254 – 261.
[44] WEIDLING I, SWERDLOW RH. Mitochondrial dysfunction and stress responses in alzheimer's disease[J]. Biology, 2019, 8(2): 39.
[45] HUANG K, TAN CY, TIAN HZ, *et al.* Exploring the bi-directional relationship between autophagy and Alzheimer's disease[J]. CNS Neuroscience & Therapeutics, 2020, 26(2): 155 – 166.
[46] GE M, CHEN S, HUANG Y, *et al.* Role of calcium homeostasis in Alzheimer's disease[J]. Neuropsychiatric Disease and Treatment, 2022, 18: 487 – 498.

(Continued on page 72)

wer side effects and enables multi-target regulation. It not only improves liver function and relieves emotional symptoms by soothing the liver and regulating qi, but also enhances sleep quality and reduce anxiety by alleviating depression and calming the mind. These characteristics highlight its dual advantages of efficacy and safety in depression treatment, making it a trending research focus in the field of antidepressants.

Conclusions

The research on the antidepressant effects of Rhizoma Cyperi originated in the late 20th century in China. In its early stages, publications on Rhizoma Cyperi for depression treatment were scarce, and progress was relatively slow. It was not until 2016 that literature on its antidepressant properties began to surge significantly. However, most studies focused on its mechanism of action, pharmacological effects, and Rhizoma Cyperi-containing compound formulas, while research on the alcohol extract of single herb Rhizoma Cyperi and its efficacy for specific depressive disorders remains limited. The exact targets and pathways of its antidepressant effects remain unclear, and its application to specific subtypes of depression is still insufficient. Therefore, further efforts are needed to explore the antidepressant mechanisms of Rhizoma Cyperi, so as to promote its application in treating more specific conditions or new therapeutic areas.

References

[1] FENG Y, XIAO L, WANG WW, *et al.* Guidelines for the diagnosis and treatment of depressive disorders in China: The second edition[J]. J Affect Disord., 2019, 253: 352 – 356.

[2] HERRMAN H, PATEL V, KIELING C, *et al.* Time for united action on depression: A Lancet-World Psychiatric Association Commission [J]. Lancet., 2022, 399(10328): 957 – 1022.

[3] MALHI GS, MANN JJ. Depression[J]. Lancet., 2018, 392(10161): 2299 – 2312.

[4] WU B, LIU XJ, QUE HQ, *et al.* Prescription rules of chinese patent drugs containing cyperus rotundus on the basis of traditional Chinese medicine inheritance support system[J]. China Pharmaceuticals, 2019, 28(13): 5 – 9. (in Chinese).

[5] ZHANG J, LIU L, XU HR, *et al.* New progress in research on chemical constituents and pharmacological action of Nutgrass Galingale Rhizome [J]. Chemical Engineer, 2021, 35(3): 55 – 57, 7. (in Chinese).

[6] XU XM, LIU WJ. Research progress of clinical application and of Cyperus rotundus[J]. Guangdong Chemical Industry, 2023, 50(9): 122 – 123, 169. (in Chinese).

[7] HARMER CJ, DUMAN RS, COWEN PJ. How do antidepressants work? New perspectives for refining future treatment approaches[J]. Lancet Psychiatry, 2017, 4(5): 409 – 418.

[8] WANG XJ, MA RY. Analysis of research hotspots of engineering cost audit based on Citespace atlas quantification[J]. Value Engineering, 2020, 39(9): 243 – 244 (in Chinese).

[9] SUN YS, QIU RR, DENG X. Research development of mapping knowledge domains in China: Analysis based on CiteSpace Ⅱ[J]. Journal of Modern Information, 2024, 34(1): 84 – 88. (in Chinese).

[10] WAN YS, ZHAO LB, LIU JY, *et al.* Trends and hot spots in health economics evaluation of bone health management: A visualized analysis based on Citespace[J]. Chinese Journal of Hospital Pharmacy, 2023,43(1): 28 – 33. (in Chinese).

[11] LIU GY, CHEN YY, WANG J, *et al.* Analysis on knowledge mapping of land and resources "One Map" based on CiteSpace[J]. Land and Resources Informatization, 2021(2): 22 – 27. (in Chinese).

[12] XU ZY, DONG ZQ. Research trend of worldwide lung cancer during recent 40 years based on bibliometric analysis[J]. Science & Technology Review, 2019, 37(22): 113 – 125. (in Chinese).

[13] LI MJ, ZHANG JQ, JIANG F, *et al.* Visualization analysis of the Zusanli (ST36) studies based on CiteSpace[J]. World Science and Technology—Modernization of Traditional Chinese Medicine and Materia Medica, 2022, 24(3): 1240 – 1252. (in Chinese).

[14] CHEN Y, CHEN CM, LIU ZY, *et al.* The methodology function of CiteSpace mapping knowledge domains[J]. Studies in Science of Science, 2015, 33(2): 242 – 253. (in Chinese).

[15] WANG LS, PAN JH. Antidepressant application of Cyperi Rhizoma and its compound formulas[J]. Lishizhen Medicine and Materia Medica Research, 2017, 28(5): 1180 – 1182. (in Chinese).

[16] LI X. Data mining-based summary of professor Li Qiyi's clinical experience in treating insomnia with comorbid depression and/or anxiety[J]. Nanjing: Nanjing University of Chinese Medicine, 2020. (in Chinese).

[17] LIU H, ZHANG ML, YU M, *et al.* Antidepressant activity evaluation and GC-MS analysis of volatile oil from vinegar-made Cyperi rhizome[J]. Drug Evaluation Research, 2020, 43(3): 436 – 442. (in Chinese).

[18] JIA HM, TANG C, LIU H, *et al.* Exploration of mechanism of antidepressant of *Cyperus rotundus* based on network pharmacology[J]. Drug Evaluation Research, 2019, 42(1): 49 – 55. (in Chinese).

[19] YUE Y, WANG PC. Development context and prospect of the new round of farmland for forest project in China based on CiteSpace visual analysis [J]. Forestry and Ecological Sciences, 2023, 38(1): 31 – 37. (in Chinese).

[20] HOU W, XU J. Research progress on pharmacological effects of Rhizoma Cyperi in different physiological systems for human body[J]. Journal of Sichuan of Traditional Chinese Medicine, 2022, 40(1): 213 – 216. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 65)

[47] GUO J, CHENG J, NORTH BJ, *et al.* Functional analyses of major cancer-related signaling pathways in Alzheimer's disease etiology[J]. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 2017, 1868(2): 341 – 358.

[48] CHANDRASEKHARAN JA, HUANG XM, HWANG AC, *et al.* Altering the anti-inflammatory lipoxin microenvironment: A new insight into Kaposi's sarcoma-associated herpesvirus pathogenesis[J]. Journal of Virology, 2016, 90(24): 11020 – 11031.

[49] HUANG SY, YANG YX, KUO K, *et al.* Herpesvirus infections and Alzheimer's disease: A Mendelian randomization study[J]. Alzheimer's Research & Therapy, 2021, 13(1): 158.

[50] SEN T, SAHA P, JIANG T, *et al.* Sulfhydration of AKT triggers Tau-phosphorylation by activating glycogen synthase kinase 3β in Alzheimer's disease[J]. Proceedings of the National Academy of Sciences, 2020, 117(8): 4418 – 4427.

[51] HAN Y, CHEN K, YU H, *et al.* Maf1 loss regulates spinogenesis and attenuates cognitive impairment in Alzheimer's disease [J]. Brain, 2024, 147(6): 2128 – 2143.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU