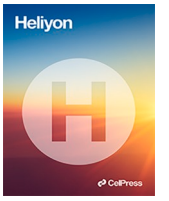
Heliyon 10 (2024) e36471

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Integrated network pharmacology and molecular docking to investigate the potential mechanism of Tufuling on Alzheimer's disease

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ARTICLE INFO

Keywords:
Tu fu Ling
Network pharmacology
Molecular docking

ABSTRACT

Objective: This study aimed to investigate the mechanism of Tu Fu Ling in treating Alzheimer's disease (AD) using network pharmacology and molecular docking.

Methods: The TCMSP and Swiss target prediction databases were utilized to confirm the active components of Tu Fu Ling and their corresponding targets, with target gene names converted using the UniProt database. Genes related to AD were collected from DisGeNET, GeneCards, and the Open Target Platform databases. Common target genes between the disease and the drug were obtained using Venny 2.1 tools and visualized using Cytoscape software. Protein-protein interaction (PPI) data were further analyzed to determine correlations between common target genes, and GO and KEGG pathway enrichment analyses were performed for intersecting genes. Finally, PYmol, AutoDock Tool, Discovery Studio 2020, and PyRx software were used for preliminary computer virtual verification and visualization of active drug ingredients and target proteins.

Results: Nine active ingredients meeting the screening criteria yielded a total of 168 genes after removing duplicates. A total of 3833 target genes were collected, with 129 overlapping target genes identified. GO enrichment analysis identified 643 biological processes, 82 cellular components, and 147 molecular functions. KEGG pathway enrichment analysis also revealed a pathway closely related to AD (hsa05010: Alzheimer's disease). In molecular docking analysis, the binding affinity between the 9 active ingredients and 10 core targets ranged from −3.5 to −12.3 kcal/mol, indicating strong binding.

Conclusion: This study preliminarily verified the combination of Tu Fu Ling's screened active ingredient and the calculated core target, suggesting a potential mechanism of action to improve the symptoms of AD patients through multi-target and multi-pathway approaches. This provides a valuable reference for further exploration of the pharmacological mechanism of AD and the formulation of drug therapy.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative condition that progresses over time, leading to cognitive dysfunction, changes in daily behavior, and memory loss. This eventually makes it one of the main contributors to dementia [1]. Despite numerous studies, the

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https://doi.org/10.1016/j.heliyon.2024.e36471
Received 29 August 2023; Received in revised form 9 August 2024; Accepted 15 August 2024
Available online 16 August 2024

etiology and pathology of AD remain poorly understood [2]. However, the main pathological features, which include the loss of neurons and synapses, the accumulation of amyloid-β (Aβ) plaques, and the formation of intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein (p-tau) [3], have been well-established. Although some drugs have received approval from the United States Food and Drug Administration (USFDA), unfortunately, they have failed to show significant improvements in clinical symptoms associated with AD [4]. Therefore, further research is essential to develop successful treatments for the effects of AD.

The Chinese Pinyin name for this herb is Tu Fu Ling, with the Latin name Smilacis Glabrae Rhizoma (SGR), also known as Smilax Glabra Roxb (SGR). It is an herbal vine that comes in both red and white varieties [5]. Tu Fu Ling has been extensively utilized in the treatment of syphilis, gonorrhea, and gout according to the Chinese Pharmacopoeia [6,7]. Moreover, the antiviral, anti-inflammatory, immunomodulatory properties, and antioxidant potential of Tu Fu Ling have been extensively researched, and it exhibits a protective effect on patients suffering from heart failure [8].

Currently, there are limited studies reporting the use of Tu Fu Ling in the treatment of AD. In this study, we leveraged the combined strengths of network pharmacology and molecular docking to predict the active ingredients and targets of Tu Fu Ling for the treatment of AD. By establishing the relationship between the drug ingredients, their targets, and the disease, we aim to provide supportive evidence for subsequent experimental research.

2. Methods

2.1. The collection of components of Tu Fu Ling

In the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, various resources are available for access, including chemicals, targets, drug-target networks, drug-target-disease networks, and more (https://www.tcmsp-e.com/tcmsp.php). The following parameters were set to collect the active components of Tu Fu Ling: a drug similarity threshold of DL $\geq$ 0.18 and blood-brain barrier permeability of BBB $\geq$ −0.3. It is important to note that BBB values range from non-penetrating (BBB- **<** -0.3) to moderate penetrating (BBB± from −0.3 to +0.3), and to strong penetrating (BBB+ **>** 0.3) [9].

2.2. Tu Fu Ling component target acquisition and gene name conversion

To ascertain the active constituents of Tufuling and their corresponding potential therapeutic targets, we referred to TCMSP and the Swiss Target Prediction database (http://www.swisstargetprediction.ch/index.php), [10]. Subsequently, we employed the UniProt database (https://www.uniprot.org/) [11], to convert the identified target genes into universally recognized gene nomenclature.

2.3. Target gene prediction of AD and intersections with drug target genes

In pursuit of target genes pertinent to "Alzheimer's disease," we conducted exhaustive searches across the DisGeNET database (https://www.disgenet.org/search) [12], the GeneCards repository (https://www.genecards.org/) [13], and the OPEN TARGET PLATFORM (https://platform.opentargets.org/), [14]. Following this, we leveraged the Venny 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/) to delineate the common targets between Alzheimer's disease and Tu Fu Ling, thereby facilitating the subsequent analysis of protein interactions.

2.4. Protein-protein interaction (PPI) network analysis

To elucidate the protein-protein interactions within the overlapping regions of Tu Fu Ling-related genes and AD-associated genes, we utilized the STRING database (https://string-db.org/) for analysis, with the species scope limited to Homo sapiens [15].

2.5. Key target screening

We imported the TSV-formatted data file retrieved from the STRING database into the Cytoscape software. We took advantage of its "cytoNCA" plugin to calculate the network's topological parameters. Degree, Closeness, and Betweenness were used as quantitative indicators to assess the importance of nodes. The resultant visualization was constructed primarily using the Degree as the basis.

2.6. Gene Ontology (GO) and KEGG pathway enrichment analysis

To conduct a further analysis of the 129 target genes, we employed the DAVID database (https://david.ncifcrf.gov/tools.jsp), [16]. We selected data sources of the human species and carried out GO and KEGG pathway enrichment analyses to obtain a comprehensive comprehension of the target genes and their associated biological functions. Subsequently, we visualized the enrichment results by using the bioinformatics database (https://www.bioinformatics.com.cn/) [17], which offers clear and intuitive figures. Additionally, we retrieved AD-related signaling pathways from the KEGG database (https://www.kegg.jp/kegg/) to better understand the molecular mechanism of Tu Fu Ling in the treatment of AD [18].

2.7. Molecular docking

To elucidate the molecular mechanism underlying the therapeutic efficacy of Tu Fu Ling in the treatment of Alzheimer's Disease (AD), we retrieved macromolecular structures in PDB format from the UniProt and RCSB Protein Data Bank (PDB) databases (https://www.rcsb.org/), [19]. Small molecule compounds were procured directly from TCMSP database. For the preparation of ligands and proteins suitable for molecular docking studies, we utilized Pymol and AutoDock Tool software to eliminate water molecules, introduce hydrogen atoms, and compute partial charges. The molecular docking experiments were performed using the vina module within the PyRx software suite. Subsequently, the results from the molecular docking simulations were visualized using GraphPad Prism and Discovery Studio 2020 clients.

3. Results

3.1. Active ingredients and targets of Tu Fu Ling

Upon the application of the defined criteria (DL $\geq$ 0.18, BBB ≥ -0.3), a total of nine active ingredients were successfully identified. Thereafter, these active ingredients' targets were meticulously consulted and predicted through the utilization of the TCMSP and the Swiss target prediction database. This comprehensive analysis yielded a total of 308 targets, which are systematically outlined and presented in Table 1.

3.2. Drug - disease intersection target and PPI analysis

After transforming the target genes of 308 active ingredients, we successfully removed 140 duplicate genes, ultimately obtaining 168 target genes to ensure the accuracy of subsequent analysis. These 168 optimized target genes overlap with the known 3833 disease target genes, resulting in 129 gene intersections, as visualized in Fig. 1. We set the minimum interaction score threshold to medium confidence (0.400) and obtained the protein interaction map from the STRING database. This map includes details such as 129 nodes, 1354 edges, and an average node degree of 21, as shown in Fig. 2.

3.3. Visualization of intersecting genes and core targets

The analysis of key target genes was conducted utilizing the cytocNCA module within the Cytoscape software. This module was instrumental in the computation and subsequent screening of genes of significance. The top 10 genes identified based on the Degree sequence, as presented in Table 2, are as follows: AKT1, SRC, TP53, ESR1, CASP3, PTGS2, BCL2, JUN, MTOR, and TGFB1.

To visualize the complex network of these genes, Fig. 3 provides a comprehensive illustration of the 129 intersecting target genes, offering a clear depiction of their interactions and relationships.

3.4. GO enrichment analysis

The data retrieved from the DAVID database was systematically organized, and an enrichment analysis was subsequently performed. A total of 643 biological processes (BP) were identified, with the top three being response to xenobiotic stimulus, positive regulation of the MAPK cascade, and the transmembrane receptor protein tyrosine kinase signaling pathway. Additionally, 82 cell components (CC) were identified, with the top three being the receptor complex, integral component of the plasma membrane, and the plasma membrane. Furthermore, 147 molecular functions (MF) were identified, with the top three being protein tyrosine kinase activity, protein serine/threonine/tyrosine kinase activity, and transmembrane receptor protein tyrosine kinase activity. Based on P-values, the top 10 with the highest degree of enrichment were selected, as depicted in Fig. 4.

3.5. KEGG pathway enrichment analysis and AD related pathway maps

Upon rigorous analysis of the data utilizing DAVID, a total of 152 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

Table 1
Information of 9 active ingredients and the number of corresponding targets.

Mol ID	Molecule Name	OB (%)	BBB	DL	Number of targets
MOL013117	4,7-Dihydroxy-5-methoxyl-6-methyl-8-formyl-flavan	37.03	−0.13	0.28	7
MOL000358	Beta-sitosterol	36.91	0.99	0.75	83
MOL000359	Sitosterol	36.91	0.87	0.75	47
MOL000449	Stigmasterol	43.83	1	0.76	72
MOL000546	Diosgenin	80.88	0.27	0.81	81
MOL005644	Palmitone	11.85	0.99	0.48	9
MOL000880	Tricosane	8.33	1.68	0.21	1
MOL013120	Octacosanal	12.19	1.02	0.42	7
MOL003894	Smilagenin	14.15	0.1	0.81	1

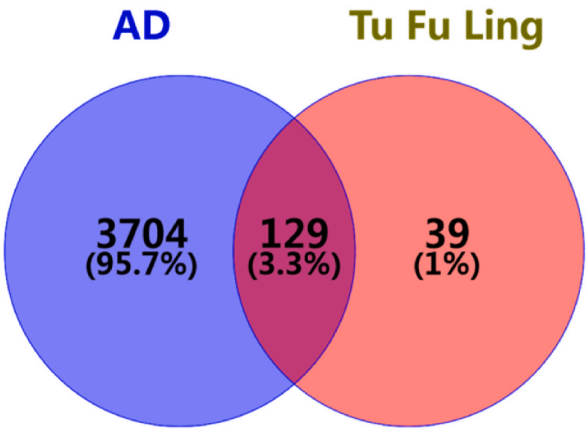

Fig. 1. Intersection targets of AD and Tu Fu Ling.

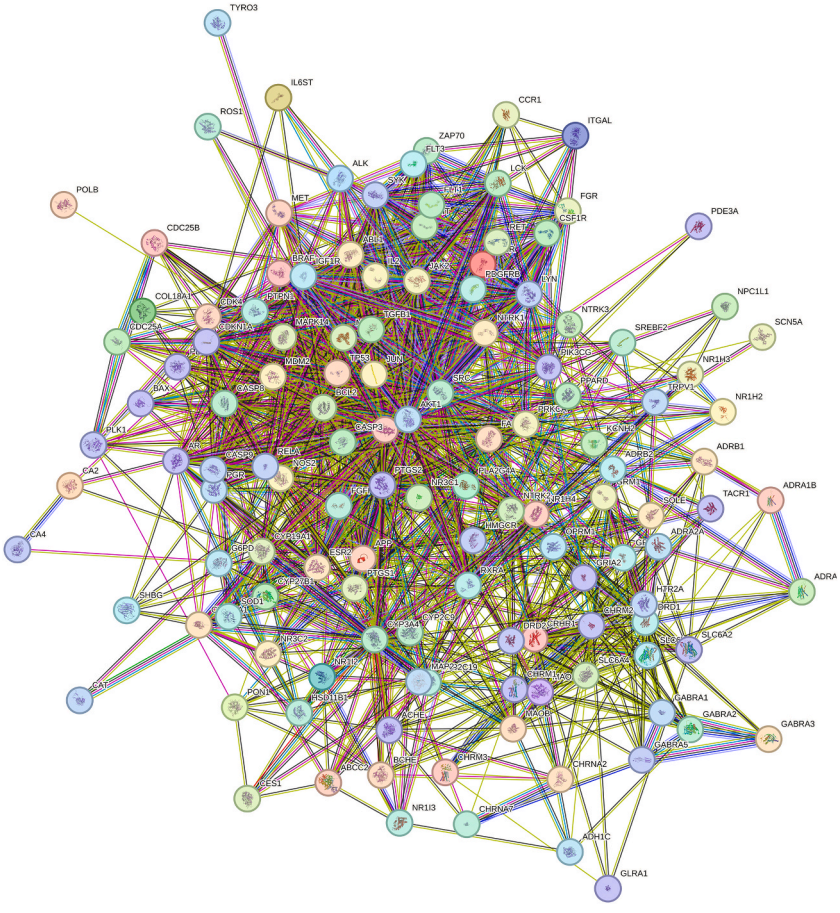

Fig. 2. number of nodes:129, number of edges:1354 average node degree:21, avg. local clustering coefficient:0.544, expected number of edges:488 PPI enrichment p-value:< 1.0e-16.

were identified. Among these, the three most prominent pathways were determined to be "Pathways in cancer," the "PI3K-AKT signaling pathway," and "Neuroactive ligand-receptor interaction," as assessed by the number of enriched genes and a significance threshold of $P < 0.05$. The first 20 pathways are presented in Fig. 5. In addition, Fig. 6 provides a visual representation of the AD-related signaling pathway, as retrieved from the KEGG database. This map highlights 15 genes that are shaded in bright pink, which include CHRM3, APP, CHRM1, NOS2, CHRNA7, INSR, BRAF, PTGS2, MTOR, RELA, CASP9, GRIN2A, CASP8, CASP3, and AKT1.

Table 2
Information of 10 key target genes.

Number	Genes	Degree	Betweenness	Closeness
1	AKT1	75	1454.1842	0.69945353
2	SRC	70	1525.2334	0.67368424
3	TP53	66	998.8347	0.6597938
4	ESR1	63	1127.1598	0.6564103
5	CASP3	60	510.09344	0.6432161
6	PTGS2	57	730.1347	0.6432161
7	BCL2	54	295.9667	0.6213592
8	JUN	54	368.64307	0.62439024
9	MTOR	47	201.23476	0.59534883
10	TGFB1	45	343.0228	0.58447486

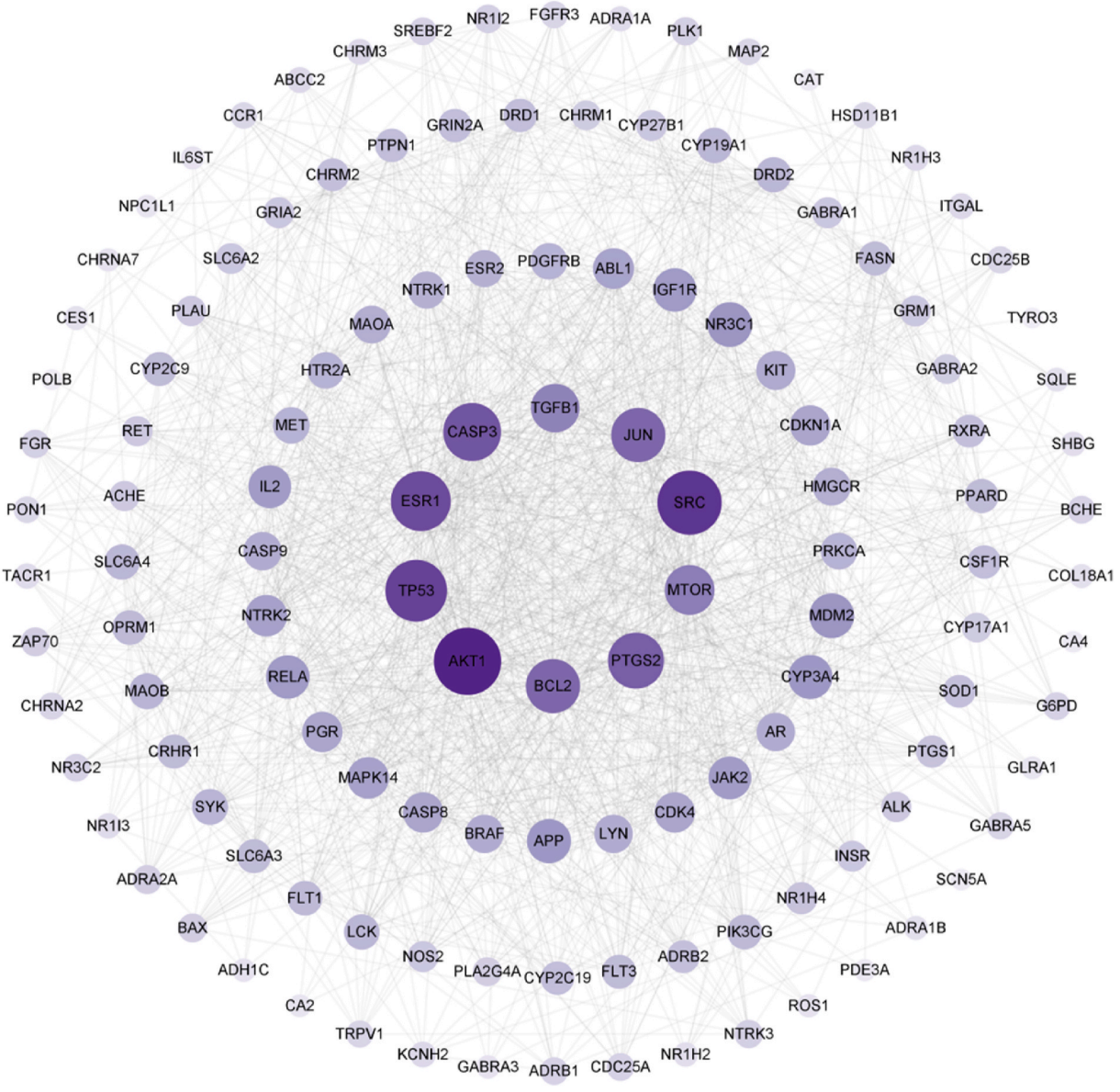

Fig. 3. The more purple the color, the larger the circle, the greater the degree value.

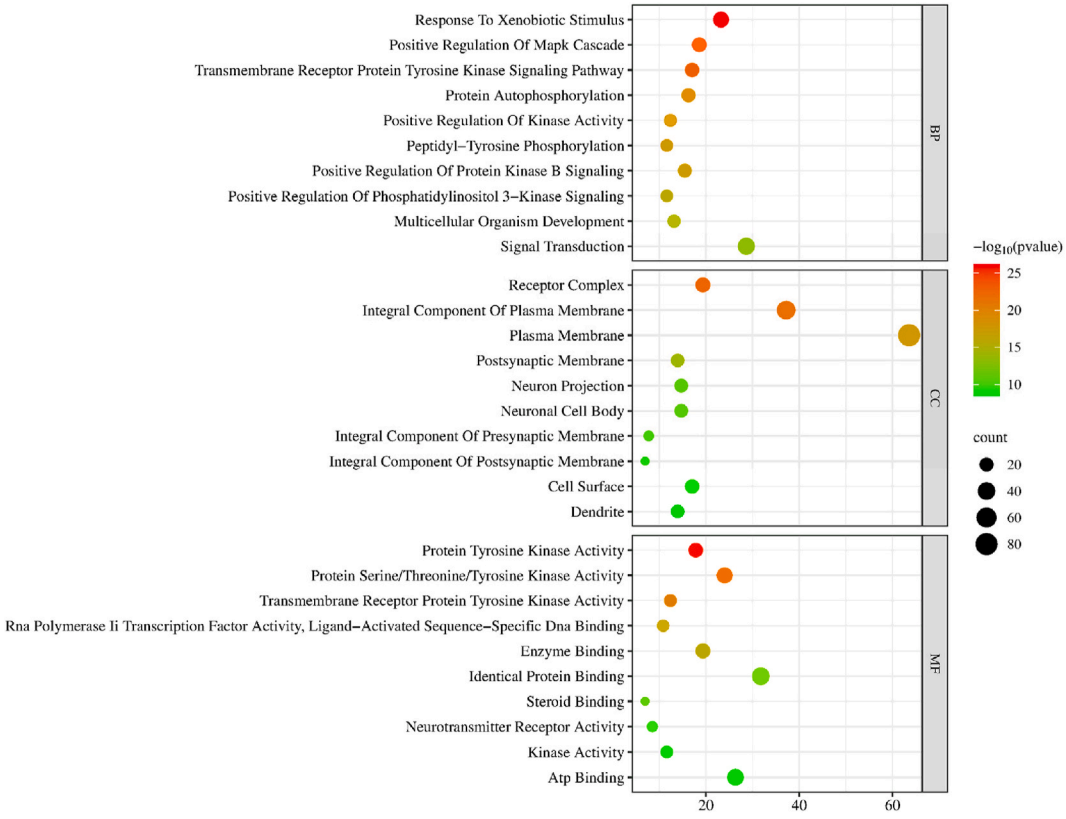

Fig. 4. Enrichment analysis: the redder the color, the smaller the P value, and the larger the circle, the more genes are enriched.

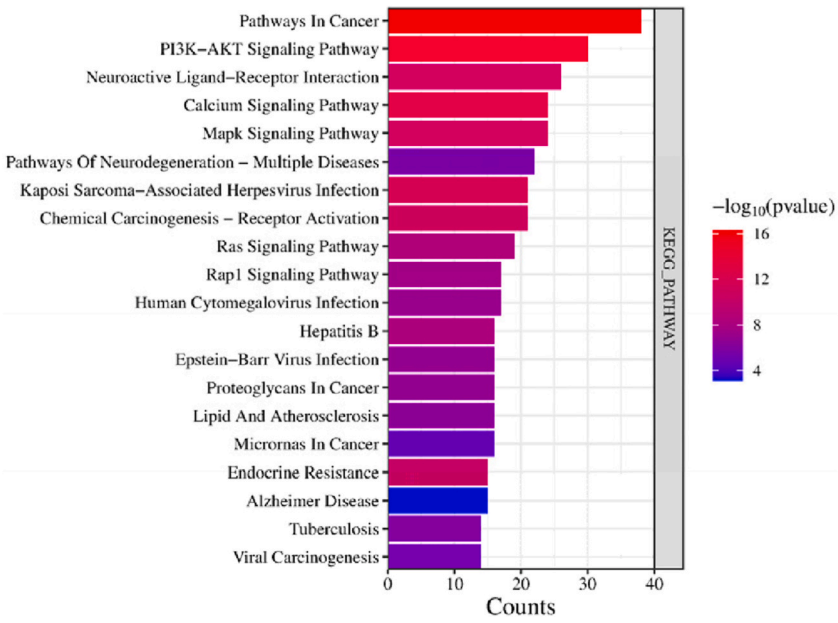

Fig. 5. Enrichment analysis of KEGG pathway.

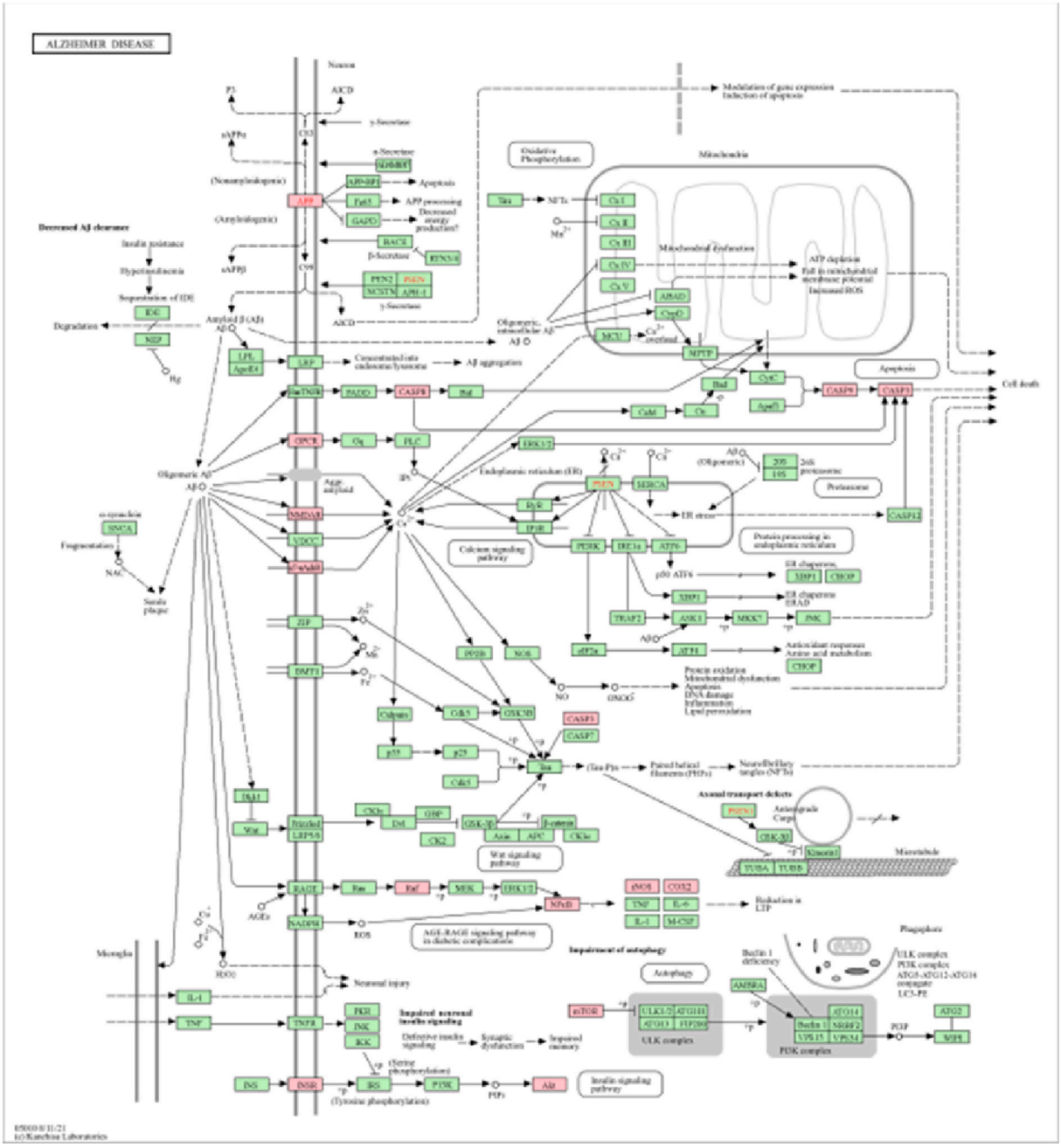

Fig. 6. The diagram of AD related signal pathway.

It is noteworthy that four of these genes—AKT1, CASP3, PTGS2, and MTOR—align with the key target genes that were identified using the cytocNCA module.

3.6. Analysis of molecular docking results

According to cytoCNA algorithm, the top 10 core targets with the highest Degree were selected for molecular docking simulation with nine Tu Fu Ling active ingredients. For more details on the two-dimensional structure of target proteins and ligands, see Table 3 and Fig. 7. The molecular docking results demonstrated the strong binding ability of these small molecules with the target proteins, with a maximum binding affinity of −12.3 kcal/mol and a minimum binding affinity of −3.5 kcal/mol. Further Binding affinities information is presented in Fig. 8. To visually illustrate the interaction between the active ingredients and macromolecular target proteins, Discovery Studio software was utilized. This software was employed to visually display the core targets AKT1, CASP3, PTGS2,

Table 3
Information about the target protein.

Number	Target	PDB_id	Resolution	UniProt entry	Reference
1	AKT1	4EJN	2.19 Å	P31749	[20]
2	SRC	1A07	2.20 Å	P12931	[21]
3	TP53	1GZH	2.60 Å	P04637	[22]
4	ESR1	1A52	2.80 Å	P03372	[20]
5	CASP3	3DEJ	2.60 Å	P42574	[23]
6	PTGS2	5F1A	2.38 Å	P35354	[24]
7	BCL2	2W3L	2.10 Å	P10415	[25]
8	JUN	1A02	2.70 Å	P05412	[22]
9	MTOR	4DRI	1.45 Å	P42345	[26]
10	TGFB1	5VQP	2.90 Å	P01137	[27]

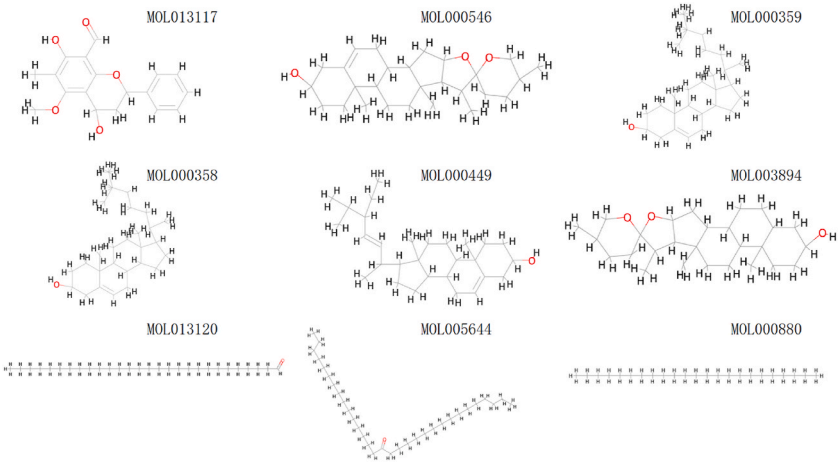

Fig. 7. 2D structure of the ligand.

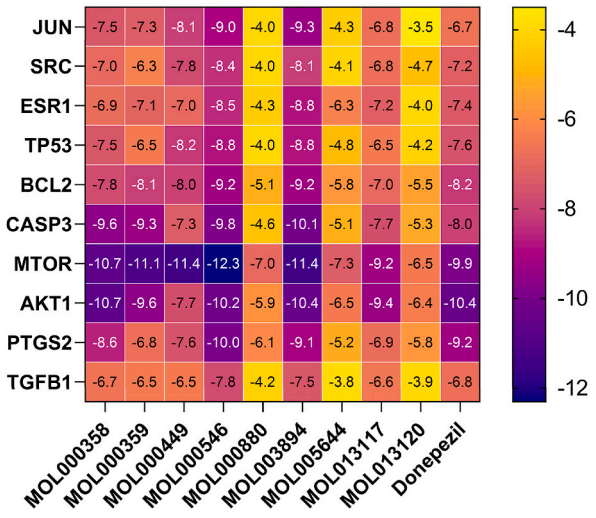

Fig. 8. Heat map of Binding affinities. The darker the color, the smaller the binding affinity, the better the binding effect.

and MTOR, which are enriched in AD-related signaling pathways from the KEGG database. As shown in Fig. 9(A–D), the highest binding energies of the aforementioned four core target points are as follows: Fig. 9A depicts CASP3 and Smilagenin; Fig. 9B presents MTOR and Diosgenin; Fig. 9C illustrates PTGS2 and Diosgenin; and Fig. 9D shows AKT1 and Beta-Sitosterol. The software analysis results indicate that these compounds interact through conventional hydrogen bonds, C–H bonds, alkyl, and pi-alkyl interactions. Software analysis revealed that these compounds exerted their main forces on protein macromolecules through conventional hydrogen

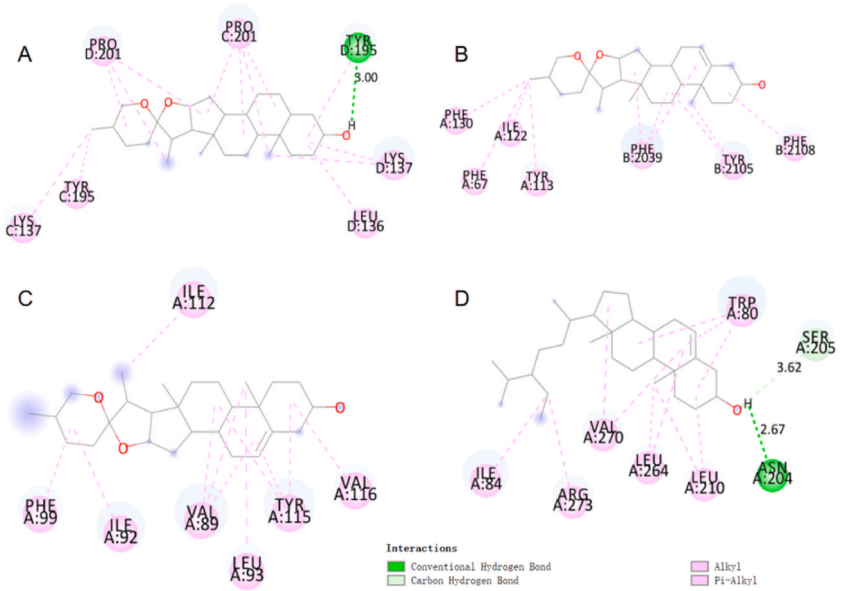

Fig. 9. 2D structure demonstration of molecular docking.

bonds, Carbon Hydrogen bonds, alkyl, and Pi-alkyl. These forces can enhance intermolecular affinity and promote stable intermolecular bonding, thereby contributing to the establishment of more robust molecular interactions.

4. Discussion

In our study, Tu Fu Ling was confirmed using filtering criteria, and we identified 9 active ingredients (4, 7-Dihydroxy-5-methoxyl-6-methyl-8-formyl-Flavan, Beta-sitosterol, Sitosterol, Stigmasterol, Diosgenin, Palmitone, Tricosane, Octacosanal, and Smilagenin). Relevant studies have shown that β-sitosterol is associated with the reduction, promotion, and improvement of Aβ deposition in the brains of APP/PS1 transgenic mice, as well as memory and learning disabilities [28]. Additionally, β-sitosterol has been demonstrated to induce blood-brain barrier permeability of PC12 cells through NGF, promoting the formation of nerve protrusions and thereby enhancing neuronal activity in the brain and alleviating AD [29]. Studies have found that Stigmasterol can inhibit the inflammation of BV2 cells induced by Aβ42 oligomers, thereby reducing neuroinflammation in APP/PS1 mice [30]. Furthermore, Stigmasterol can reduce the production of ROS in SH-SY5Y cells, up-regulate the level of apoptotic protein (Bcl-2), protect cells from oxidative stress-induced cell death, and prevent neurodegenerative diseases [31]. Another active ingredient we identified, Diosgenin, has been studied for its relationship with AD and found to significantly improve degenerative axonal function and memory function in 5XFAD mice with axonal degeneration and memory impairment [32]. In addition, Diosgenin has been shown to reduce the expression level of HSC70 in neurons treated with Aβ and induce axon regeneration [33]. Studies have shown that Smilagenin may improve the learning and memory ability of AD mice by inhibiting the activity of HDAC or increasing the expression of P300 protein to promote the level of histone acetylation in the promoter region of the BDNF gene [34].

According to the results of the top 10 Go enrichment analysis, the 129 intersection genes of Tu Fu Ling and AD are primarily concentrated in cell membranes. These genes may regulate a variety of biological processes through protein tyrosine kinase activity, protein serine/threonine/tyrosine kinase activity, and transmembrane receptor protein tyrosine kinase activity in order to treat diseases. According to the KEGG enrichment analysis, the pathway closely associated with AD (hsa05010:Alzheimer disease) was enriched, with AKT1, CASP3, PTGS2, and MTOR genes intersecting with the core targets obtained from module calculation. Studies have shown that treating 5xFAD transgenic mice with SC79 can activate Akt1, alleviating long-term memory decline and improving altered anxiety levels in AD mouse models [35]. Dysregulation of Akt1 and mTOR signaling in APP/PS1 mice may lead to behavioral deficits and altered synaptic plasticity, which appear to be age- and sex-related [36]. Caspase 3, encoded by CASP3, promotes α-synuclein interneuronal transmission and causes neuronal cell death once activated [37]. Western blotting experiments showed significantly increased expressions of EGFR and CASP3 in 3 × Tg-AD mice compared to the WT group, indicating that Chuanxiong Renshen decoction (CRD) could treat AD by down-regulating the expression of CASP3 and EGFR [38]. RT-qPCR analysis of peripheral blood samples from 10 AD patients and 10 healthy volunteers revealed lower mRNA expression levels of PTGS2 and TOMM20 in the AD group compared to the control group, while the expressions of OPTN and VDAC1 were up-regulated. These four genes have potential as diagnostic and prognostic indicators of AD [39]. Studies on SH-SY5Y cells treated with Aβ25-35 and peripheral blood of AD patients showed that the expression level of miR-26a-5p was significantly up-regulated, while the expression level of PTGS2 was significantly down-regulated [40]. Nuclear translocation of TFEB is reduced in the hippocampus of T2DM mice and in HT22 cells cultured with high glucose (HG), while activation of MTOR-dependent TFEB at the post-translational level and overexpression of

neuron-targeted TFEB at the transcriptional level promote the clearance of ALP-targeted AD-related proteins such as Aβ and p-Tau [41]. MicroRNA-128 can promote autophagy by directly targeting mTOR, thereby enhancing Aβ clearance and reducing Aβ levels [42].

The molecular docking analysis indicates that the binding affinity of the active component ranges between −3.5 and −12.3 kcal/mol, suggesting its good binding capability with 10 core targets. Specifically, the binding affinity of CASP3 with smilagenin is −10.1 kcal/mol, MTOR with diosgenin is −12.3 kcal/mol, PTGS2 with diosgenin is −10 kcal/mol, and AKT1 with beta-sitosterol is −10.7 kcal/mol. When compared with the positive control drug donepezil, donepezil shows a higher binding affinity to core target proteins than the active components Palmitone (MOL005644), Tricosane (MOL000880), and Octacosanal (MOL013120). In addition, AKT1, MTOR, PTGS2, and CASP3 are among the top five proteins with the highest binding affinity to donepezil. Therefore, it can be speculated that these core targets may be the therapeutic targets of Tu Fu Ling in the treatment of AD. However, this study has some limitations that need to be further investigated using cell and animal models.

5. Conclusion

This study used network pharmacology to predict and identify nine active ingredients from Tu Fu Ling. Molecular docking technology was then employed to verify the feasibility of these active ingredients binding to AD targets. Although there are differences in binding affinity between our active ingredients and donepezil at specific targets, our active ingredients demonstrate potential for binding at multiple core targets. Specifically, β-sitosterol, stigmasterol, diosgenin and Smilagenin play crucial roles in inhibiting ROS generation, reducing Aβ deposition, alleviating neuroinflammation, and promoting axonal growth. These functions may contribute to improving the symptoms of AD patients through multiple targets and pathways. Overall, this work provides a foundation for more in-depth mechanistic studies and drug development efforts toward multi-target therapies for AD.

The limitations and shortcomings of this study include

The results lacked experimental validation, being based only on computational prediction for 9 major components without testing other minor ingredients.

Supported by

Key project of the special fund for basic research of local undergraduate colleges and universities granted by Department of Science and Technology of Yunnan Province(NO. 202101AN070028)

Data availability statement

The data information for this article can be obtained from public databases and supplementary materials.

CRediT authorship contribution statement

Ziyou Zhang: Writing – original draft, Visualization, Validation, Software. **Jiamao Cheng:** Writing – original draft, Visualization, Validation, Project administration. **Xinpei Zhou:** Writing – original draft, Visualization, Validation, Data curation. **Haoyi Wu:** Data curation. **Bensi Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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