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Original Article

Mechanism of Tianma Gouteng Decoction in the treatment of Parkinson's disease based on network pharmacology and molecular docking

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Abstract: Objective: To explore the pharmacological mechanism and molecular targets of Tianma Gouteng Decoction (TMGTD) in the treatment of Parkinson's disease (PD). Methods: We applied network pharmacology to screen the active components of TMGTD and predict target genes in multiple Chinese herbal medicine databases and compound databases, and built a drug-ingredient-target network. Then, we used the CytoHubba plug-in to filter out the core components of TMGTD according to the order of degree value. We screened PD-related pathogenic targets in the DrugBank, Genecard and OMIM databases from high to low in Betweenness Centrality (BC) value and Closeness Centrality (CC) value. Subsequently, we determined the intersection target of TMGTD and PD by Venn diagram and performed protein-protein interaction (PPI) analysis, Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on core molecules and intersection targets. Finally, molecular docking was performed to verify the binding of the top three core molecules of TMGTD with the top three core targets of PD. Results: The core components of TMGTD are quercetin, kaempferol and palmitic acid. The main targets of TMGTD in the treatment of PD are ALB, GAPDH and AKT1. GO analysis and KEGG analysis showed that the biological process of TMGTD in the treatment of PD is closely related to the activities of neurotransmitter receptors, G protein-coupled receptors and dopamine neurotransmitter receptors. TMGTD possesses therapeutic effects on PD mainly through the PI3K-Akt signaling pathway and MAPK signaling pathway. Molecular docking shows the high affinity of the quercetin, kaempferol and palmitic acid with PD core targets. Conclusion: TMGTD plays a pivotal role in the treatment of PD through multiple components, multiple targets and multiple pathways. The results provide a research direction for the subsequent exploration of the mechanism of TMGTD in PD treatment.

Keywords: Network pharmacology, Tianma Gouteng Decoction, Parkinson's disease, molecular docking, mechanism

Introduction

The incidence rate of Parkinson's disease (PD) is increasing annually [1]. Governments and organizations around the world have invested a lot of human and financial resources in the treatment of PD. PD is mainly characterized by motor symptoms such as static tremor, abnormal gait and posture, myotonia, and motor retardation, accompanied by non motor symptoms such as constipation, anosmia, mental and cognitive abnormalities. With the progression of PD, the patient's motor and non motor symptoms gradually worsen, thereby severely restricting their daily life.

At present, the treatment of PD is mainly depends on drugs and surgery, of which drugs are the main means to improve the motor symptoms and delay the disease progress. Drugs with different mechanisms of action are selected according to the clinical characteristics of patients. Levodopa [2], dopamine receptor agonists [3], monoamine oxidase type B inhibitor

[4], catechol-o-methyltransferase inhibitor [5], anticholinergic drugs [6] and amantadine [7] are the commonly used drugs. These drugs are effective in the early stages of PD, but with disease progression and increased drug dosage, the sensitivity of patients to drugs gradually decreases, and serious complications occur. Surgical treatment is a supplementary therapy for PD when the effect of drug treatment is not good. With the globus pallidus internus (GPI) and subthalamic nucleus [5] as the targets, Deep brain stimulation (DBS) [7] is the most common surgery for PD.

In addition, physiotherapy plays an active role in the treatment of PD through improving the gait and balance and other functional activities [8]. The treatment of non motor symptoms [9] and psychological intervention [10] of PD have also been given more and more attention. The treatment of PD is comprehensive, but it is still important to find effective and sustainable alternative therapies.

In China, many PD patients turn to traditional Chinese medicine (TCM). TCM, with its self-contained theory and treatment method, has been shown to be effective for PD patients In terms of alleviating clinical symptoms. Tianma Gouteng Decoction (TMGTD), a TCM formula, plays a neuroprotective role in PD modeled animals and cells [11]. It contains 11 ingredients including Gastrodia elata Bl, Uncariae Ramulus Cumuncis, Cassia obtusifolia, Scutellariae Radix, Gardeniae Fructus, Leonuri Herba, Cyathula officinalis Kuan, Eucommiae Cortex, Caulis Ploygoni multiflori, Poria cum radix pini and Taxillus herba. Modern pharmacological studies have shown that TMGTD inhibits the apoptosis of dopaminergic neurons by resisting oxidative stress, increasing BCL-2 expression and inhibiting Bax activation [12]. In addition, TMGTD could partially restore the protein levels of vasoactive substances TXB2, ET and 6-keto-PGF1alpha in PD mouse models [13]. Clinical studies have shown that TMGTD is effective in improving limb tremor in PD patients [14]. However, the molecular mechanism behind the role of TMGTD in PD treatment is still not clear.

Network pharmacology is a high-throughput omics data analysis method based on information retrieval of network databases on a public platform. It combines biology and pharmacology theories and provides a new research strategy for studying the mechanism of action of TCM. In order to clarify the main pharmacological mechanism of TMGTD, this study used network pharmacology to predict the core active ingredients of TMGTD for PD treatment and built a drug-component-target network. In order to verify the main pharmacological action mechanism of TMGTD, the core molecules and targets of TMGTD for the treatment of PD was selected and its biological mechanism of action and signal pathway through protein-protein interaction (PPI) analysis, Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were obtained. Finally, molecular docking was carried out to confirm its molecular mechanism in the treatment of PD. This study provides the direction and basis for the follow-up laboratory research of TMGTD in the treatment of PD. The framework of this study is illustrated in **Figure 1**.

Materials and methods

Screening of potential active components and related target genes in TMGTD

The compounds and corresponding target information of 11 components in TMGTD were collected from the following databases, namely Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php) [15]. The Encyclopedia of Traditional Chinese Medicine (ETCM) (http://www.tcmip.cn/ETCM/index.php/Home/Index/) [16]. In order to consider the changes of drug absorption and utilization in the human body, pharmacokinetic data including oral bioavailability (OB) ≥30% and drug likeness (DL) ≥0.18 were set up. Then, the main components of TMGTD and its target proteins were collected.

In order to clarify the absorption and distribution of the main active components of TMGTD, the pharmacokinetic information of the core components was predicted by ADMETlab 2.0 (https://admetmesh.scbdd.com/), and the absorption, distribution, metabolism and excretion of the drug components were predicted. ADMETlab 2.0 is a public website providing pharmacokinetic and toxicity predictions [17].

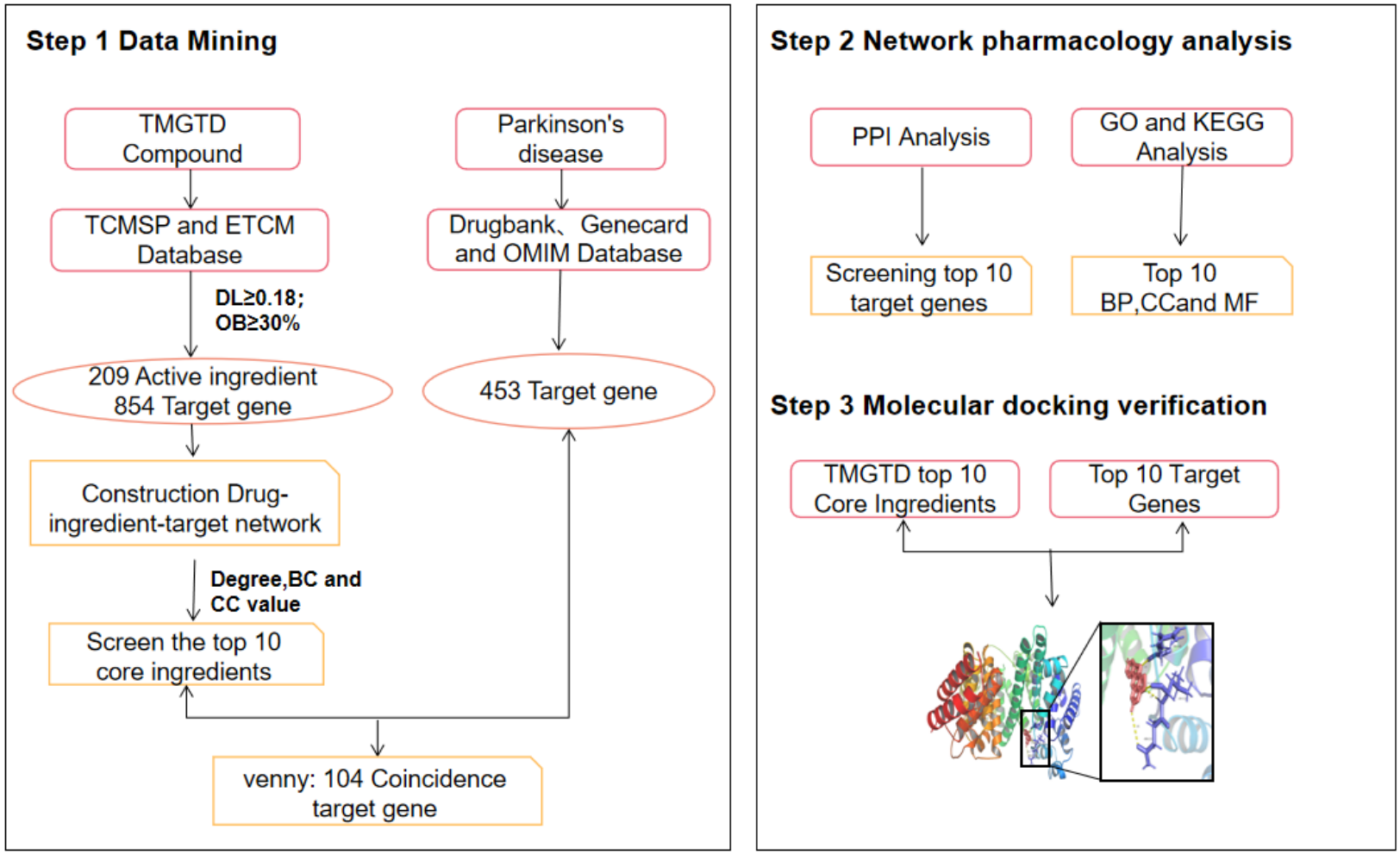


Figure 1. Workflow of network pharmacology analysis.

Screening for Parkinson's disease-related targets

PD-related targets were screened from the following 3 databases, namely Drugbank (https://go.drugbank.com/) [18], genecard (https://www.gene-cards.org/) [19] and OMIM (https://omim.org/) [20]. The keywords "Parkinson's disease" were entered into the database search box, and targets of the three databases were pooled in each database, then, merged and de-duplicated. The processed targets were the PD targets used in this study.

Construction of drug-ingredient-target network and identification of core component target network

Before building the network, the drug targets and disease targets collected above were brought into the UniProt database [21], the species was set as "Homo sapiens", and the target names were standardized to obtain the duplicate free UniProt ID and gene name. Subsequently, the drug and disease target data sets were brought into the Venn database (https://bioinfogp.cnb.csic.es/tools/venny/), and the obtained overlapping targets were the candidate active targets for TMGTD against PD. Then, Cytoscape software was used to construct the drug-active ingredients-target network, in which "drug" was set as a circle, "candidate active ingredient" was set as a triangle, drug common ingredient was set as a square, and "target" was set as a diamond. Then we calculated the node degree in network through the Cytohubba plug-in [22], screened the value which is twice the median degree value for the nodes, and found the core components and targets of TMGTD for PD treatment from high to low.

Protein-protein interaction (PPI) analysis

In order to obtain the information of protein-protein interaction network, the overlapping targets obtained from the above Venn database were imported into the string database (https://cn.string-db.org/) [23]. We set the organism as "Homo sapiens" and the required minimum interaction score as "highest confidence >0.9" [24]. The PPI network was constructed by Cytoscape 3.8.2. The degree, Betweenness Centrality (BC) and Closeness Centrality (CC) parameters of each node in the network were calculated, and the node attributes in the PPI network were analyzed [25]. In the study, the target nodes with high BC and CC values were selected to further study the interaction network relationship between core proteins and to explore the direct physical interaction and indirect functional relationship between core proteins.

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

In order to further explore the related biological processes (BP), molecular functions (MF), cellular components (CC) [26] and regulatory signal transduction pathways [27] involved in the core targets of TMGTD in the treatment of PD, the candidate core target information was brought into DAVID database (DAVID version 6.8, https://david.ncifcrf.gov/.2022-8-10) for GO analysis and KEGG enrichment analysis. The screening parameters were set as $P < 0.01$, FDR < 0.05 [28] and the obtained GO and KEGG data were adjusted to the required format. Then, we uploaded the data to Bioinformatics (http://www.bioinformatics.com.cn/) for visualization.

Molecular docking verification

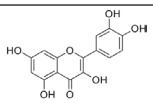
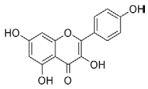
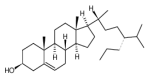
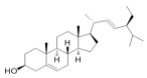
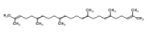
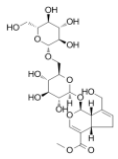
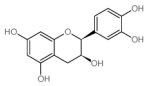
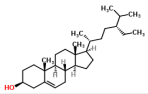
Through autodock 4.2.6 software, the molecular docking between active drug components and hub genes was verified, and the geometric matching and energy matching processes were simulated [29, 30]. The suitable proteins structure of potential active targets were obtained from the PDB protein database [31] (https://www.rcsb.org/). Auto dock tools (version 1.5.7) and MOE were used to prepare proteins and ligands. The target proteins and ligands were treated by removing water molecules and replacing them with hydrogen, then the docking of the target proteins and ligands were simulated, and the minimum binding energy was calculated. Finally, the optimal position image of the docking of the protein and ligands were visualized in PyMOL software.

Results

Screening of TMGTD-active compounds and their potential targets

A total of 209 active ingredients were screened out from TMGTD, among which 28 were

Table 1. Active Ingredient Information of Tianma Gouteng Decoction (TMGTD)

Active compound	Molecule structure	Degree	Herb
quercetin		1026	Uncariae Ramulus Cumuncis, Taxillus herba, Gardeniae Fructus, Leonuri Herba, Cyathula officinalis Kuan, Eucommiae Cortex, Caulis Ploygoni multiflori
kaempferol		357	Uncariae Ramulus Cumuncis, Gardeniae Fructus, Leonuri Herba, Eucommiae Cortex, Caulis Ploygoni multiflori
Palmitic acid		305	Gastrodia elata Bl, Cassia obtusifolia, Poria cum radix pini
beta-sitosterol		234	Gastrodia elata Bl, Uncariae Ramulus Cumuncis, Scutellariae Radix, Gardeniae Fructus, Cyathula officinalis Kuan, Eucommiae Cortex
Stigmasterol		64	Scutellariae Radix, Gardeniae Fructus
Supraene		8	Scutellariae Radix, Gardeniae Fructus
GBGB		18	Gardeniae Fructus, Eucommiae Cortex
ent-Epicatechin		21	Uncariae Ramulus Cumuncis, Scutellariae Radix, Eucommiae Cortex
sitosterol		12	Uncariae Ramulus Cumuncis, Taxillus herba, Scutellariae Radix

Gastrodia elata Bl, 32 were Uncariae Ramulus Cumuncis, 4 were Cassia obtusifolia, 36 were Scutellariae Radix, 20 were Gardeniae Fructus, 8 were Leonuri Herba, 4 were Cyathula officinalis Kuan, 33 were Eucommiae Cortex, 11 were Caulis Ploygoni multiflori, 31 were Poria cum radix pini and 2 were Taxillus herba. A total of 187 compounds with different components were found in the compound recipes, and 854 non-repetitive target genes were identified through the TCMSP database combined with the Swiss Target Prediction tool. **Table 1** presents the overlapping active ingredient information from TMGTD.

We constructed a TMGTD drug-component-target network with 1147 nodes and 5440 edges, as shown in **Figure 2**. The light purple diamond in the middle represents the potential target gene; the dark blue square is the common component of the Chinese medicine in the compound; the yellow ellipse is the medicine of the TMGTD compound; the triangles surrounding 11 TCM represent the corresponding active ingredients of each medicine, of which the light pink is the active ingredient of Cyathula officinalis Kuan, the flesh pink is the active ingredient of Eucommiae cortex, the dark pink is the active ingredient of Poria cum Radix pini, the orange red is the active ingredient of Uncariae Ramulus cumuncis, and the dark green is the active ingredient of Scutellaria radix, the orange is the active ingredient of Cassia obtusifolia, the light green is the active ingredient of Gastrodia elata Bl, the lake green is the active ingredient of Caulis ploygoni Multiflori, the sky blue is the active ingredient of Leonuri Herba, and the grass green is the active ingredient of Gardeniae Fructus. The active ingredients of Taxillus herba are the common ingredients quercetin and sitosterol. According to the degree value, BC value and CC value, the top 10 core active ingredients were determined in **Table 2**, indicating that quercetin, kaempferol, Palmitic acid are the main active ingredients in TMGTD.

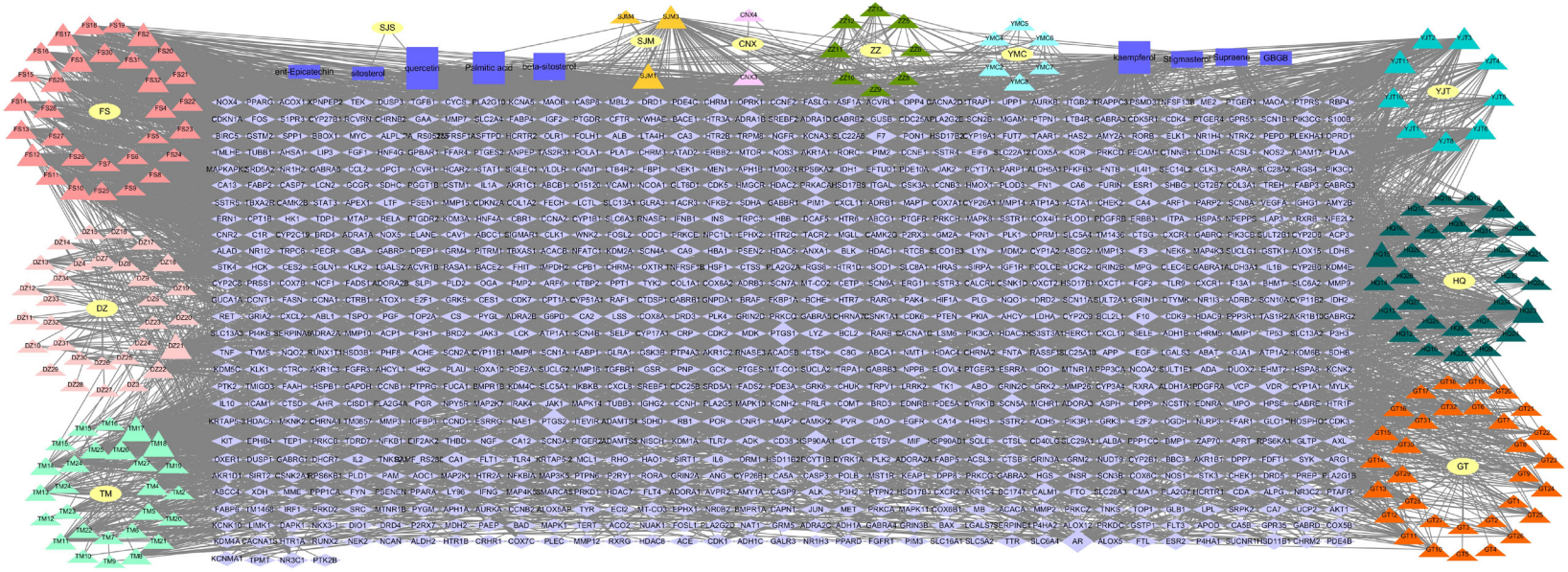
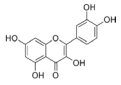
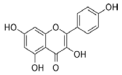
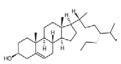
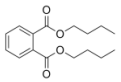
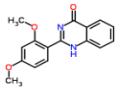
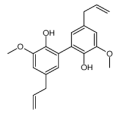
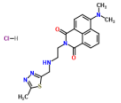
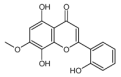
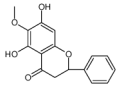


Figure 2. Tianma Gouteng Decoction (TMGTD) drug-ingredient-target network diagram. (The light purple diamonds represent potential targets of TMGTD; the yellow circles represent TMGTD drug composition; the dark blue squares represent TMGTD drug common components; and the triangles represent active ingredients of each drug).

Table 2. Top 10 active ingredients of TMGTD

Active compound	Molecule structure	Degree	Betweenness Centrality	Closeness Centrality	Herb
Quercetin		1026	0.18958816	0.40840517	Uncariae Ramulus Cumuncis, Taxillus herba, Gardeniae Fructus, Leonuri Herba, Cyathula officinalis Kuan, Eucommiae Cortex, Caulis Ploygoni multiflori
kaempferol		357	0.07010061	0.38594705	Uncariae Ramulus Cumuncis, Gardeniae Fructus, Leonuri Herba, Eucommiae Cortex, Caulis Ploygoni multiflori
Palmitic acid		305	0.11271945	0.38752556	Gastrodia elata Bl,Cassia obtusifolia, Poria cum radix pini
beta-sitosterol		234	0.02350271	0.35049322	Gastrodia elata Bl, Uncariae Ramulus Cumuncis, Scutellariae Radix, Gardeniae Fructus, Cyathula officinalis Kuan, Eucommiae Cortex
Dibutylphthalate		117	0.08307299	0.36026616	Cassia obtusifolia
β-1,3-glucanase		112	0.08252282	0.36867704	Gastrodia elata Bl
Dehydrodieugenol		110	0.07266879	0.35398506	Eucommiae Cortex
Chitinase		103	0.07920179	0.36606568	Gastrodia elata Bl
5,8,2'-Trihydroxy-7-methoxyflavone		101	0.02575221	0.36891629	Scutellariae Radix
dihydrooroxylin A		91	0.05051438	0.35754717	Scutellariae Radix

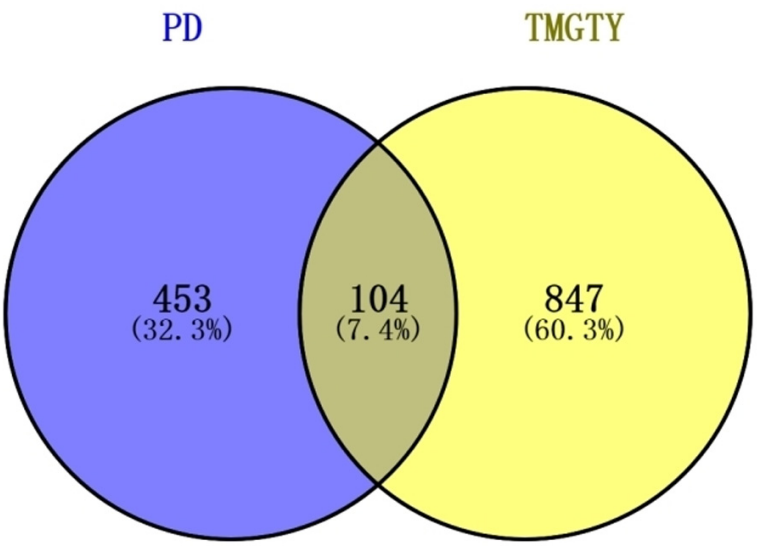


Figure 3. Venn diagram of intersection target genes of TMGTD (drug) for PD (disease). There were 104 overlapping candidate targets between TMGTD and known targets associated with the pathological process of PD.

Screening of core targets of TMGTD in the treatment of PD

The PD genes were identified through Drugbank, genecard and OMIM databases. After removal of the duplicate values, a total of 453 PD-related target genes were found. Through the Venn diagram results, it was found that there are 104 common targets between TMGTD targets and PD targets (as shown in **Figure 3**).

PPI network construction and topological clustering analysis of hub genes

We brought 104 TMGTD and PD intersection targets into the String database and obtained protein interaction network data. Then we imported them into Cytoscape 3.7.2 software for visualization and core network screening, as shown in **Figure 4**. There were 104 overlapping proteins to construct a PPI network. The main parameters of network contained the number of nodes (104) and edges (2404).

In this study, in order to screen out the core PPI-acting proteins, we set the parameters that proteins with degree value, BC value and CC value were all greater than the average. The parameters were degree value >21, BC value >0.004, and CC value >0.523. The core PPI network contained 41 nodes and 1554 edges.

To illustrate the effect of TMGTD on the core targets of PD, MCODE was used to analyze the core PPI network. There are and the identified targets were divided into 7 groups, representing potential key targets in PD therapy, as shown in **Figure 4C**. At the same time, we screened the top 10 targets according to the Degree value, BC value and CC value, as shown in **Table 3**. The node degree of proteins in the top 10 were ALB, GAPDH, AKT1, INS, TNF, APP, CASP3, IL6, TP53, IL1B.

GO enrichment and KEGG pathway analysis

Enrichment analysis was performed using DAVID, and the data generated by GO enrichment analysis were sorted by $P < 0.01$, FDR < 0.05 entries, including 250 biological processes (BP), 63 cellular components (CC) and 44 molecular functions (MF). Sorted by Lg *P* value, the top 10 entries in the BP, CC and MF data were selected for visualization, as shown in **Figure 5**.

Biological processes are associated with modulation of neurotransmitter levels, neuronal death, chemical synaptic transmission and membrane potential modulation, cognitive learning and memory. Cellular components are closely related to synaptic membranes and neuronal cell bodies. Molecular functions are reflected in neurotransmitter receptor activity, G protein-coupled receptor activity, and dopamine neurotransmitter receptor activity, as well as ammonium ion binding, catecholamine binding, dopamine binding, neurotransmitter binding, hormone binding, amine binding, and serum element binding.

Through the KEGG pathway enrichment analysis data in the David database, a total of 55 entries with $P < 0.01$ and FDR < 0.05 were screened out. According to the *P* value, the top 20 entries were selected for bubble chart drawing, as shown in **Figure 6**. KEGG analysis

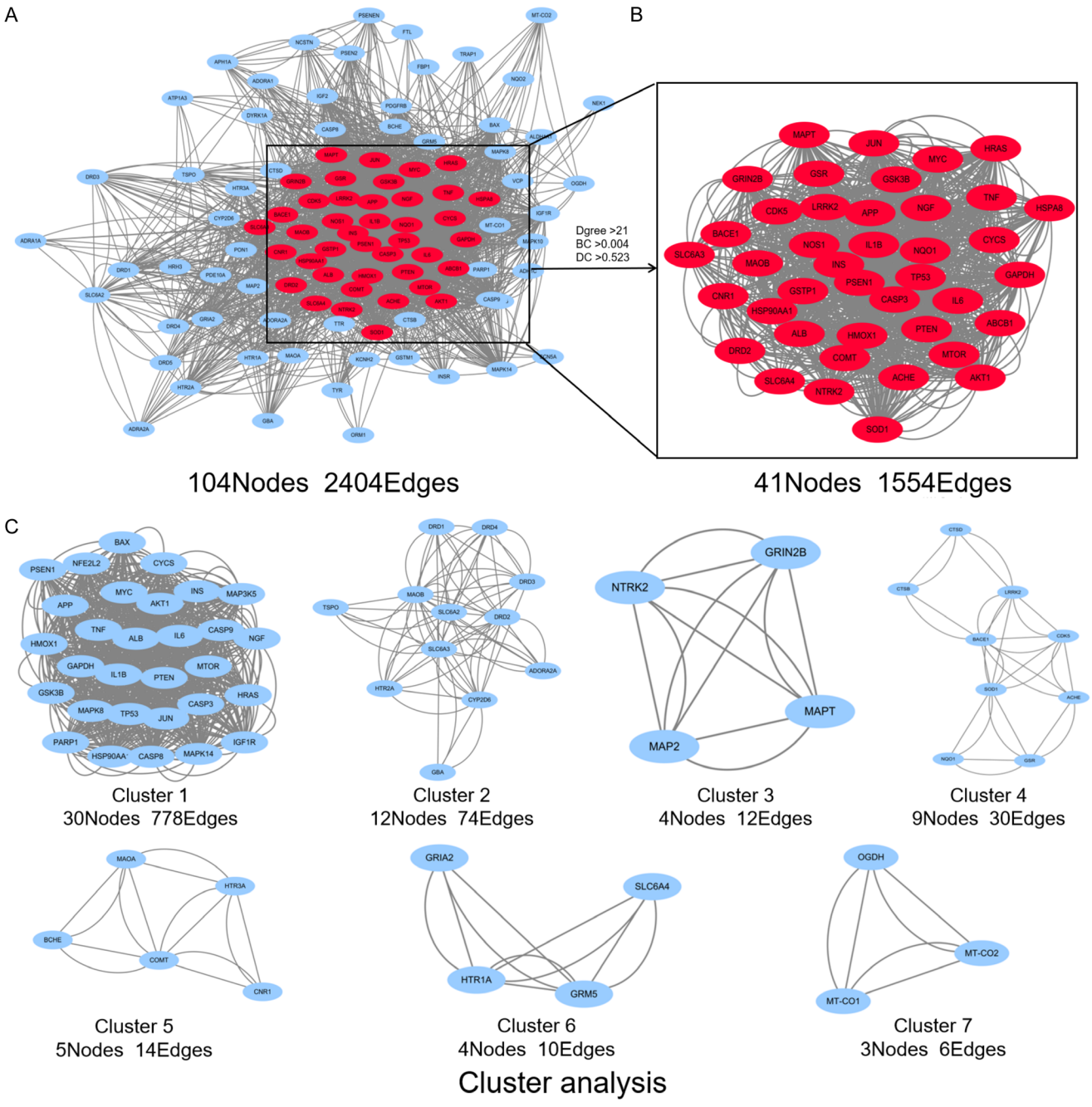

A
B
Dgree >21
BC >0.004
DC >0.523
104Nodes 2404Edges
41Nodes 1554Edges
C
Cluster 1
30Nodes 778Edges
Cluster 2
12Nodes 74Edges
Cluster 3
4Nodes 12Edges
Cluster 4
9Nodes 30Edges
Cluster 5
5Nodes 14Edges
Cluster 6
4Nodes 10Edges
Cluster 7
3Nodes 6Edges
Cluster analysis

Figure 4. Identification of potential targets of TMGTD for the treatment of PD by protein-protein interaction (PPI) analysis. A. The PPI network of intersection targets between TMGTD and PD was analyzed by String database. B. Determine the core target network by screening greater than Degree value, Betweenness Centrality (BC) and Closeness Centrality (CC) median values. C. Through the cluster analysis of the MCODE plug-in, 7 groups of core action groups were identified.

Table 3. Top 10 core target information of protein-protein interaction (PPI) network

Target name	Protein name	Degree	Betweenness Centrality	Closeness Centrality
ALB	Albumin	65	0.08229507	0.73722628
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	60	0.06320935	0.70629371
AKT1	RAC-alpha serine	57	0.03559661	0.69655172000
INS	Insulin	55	0.03819642	0.68707483
TNF	Tumor necrosis factor	53	0.0302108	0.67785235
APP	Amyloid-beta precursor protein	52	0.03933287	0.66887417
CASP3	Caspase-3	51	0.01645894	0.66447368
IL6	Interleukin-6	50	0.02569663	0.66013072
TP53	Cellular tumor antigen p53	49	0.0180041	0.62732919
IL1B	Interleukin-1beta	47	0.01460551	0.6474359

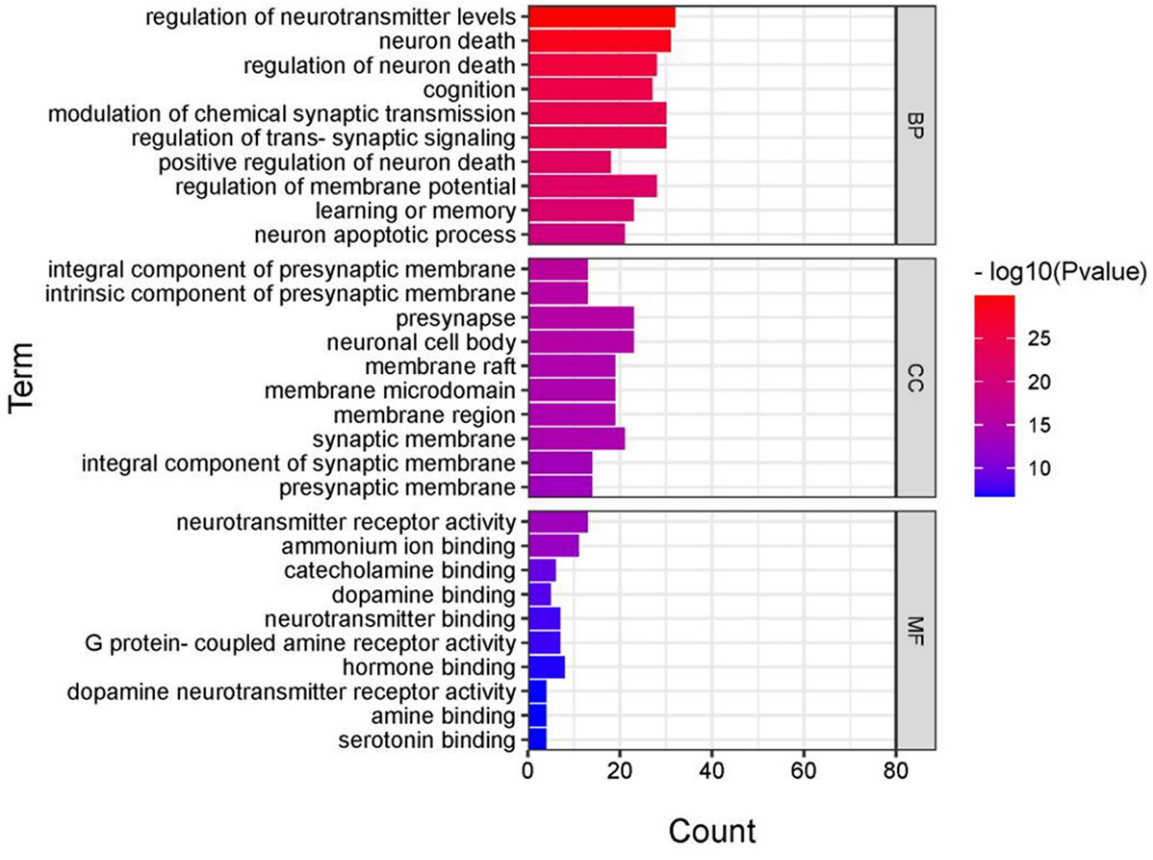


Figure 5. The Gene Ontology (GO) function and KEGG pathway enrichments. Top-10 items of biological process (BP), cellular component (CC) and molecular function (MF) of GO analysis. Set the parameters "*P* value < 0.01, FDR value < 0.05".

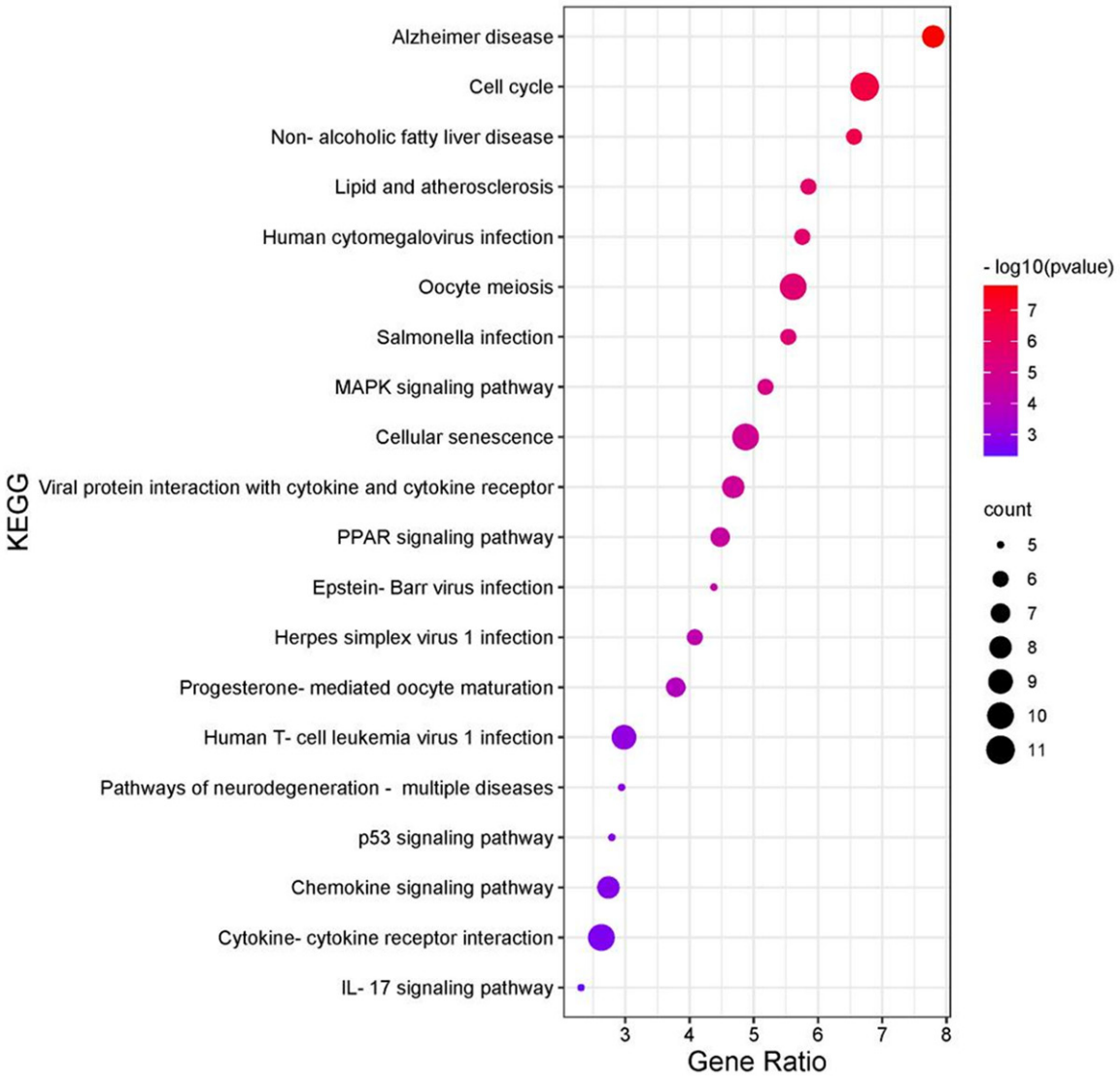


Figure 6. Top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of hub genes. The abscissa shows the enrichment factor, and the ordinate shows the KEGG pathways. The color of the dot represents the adjusted *P* value, and the size of the dot represents the number of core targets mapped to the reference pathways.

showed that it was highly related to PI3K-Akt signaling pathway and MAPK signaling pathway.

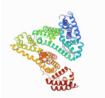
Molecular docking verification

According to the results of network pharmacology analysis, we analyzed the combination ability of top 3 active components of TMGTD, quercetin, kaempferol, and Palmitic acid, with the top 10 target proteins ALB (PDB ID: 4L8U), AKT1 (PDB ID: 1UNQ), APP (PDB ID: 1TKN), CASP3 (PDB ID: 3H0E), GAPDH (PDB ID: 6M61), IL1B (PDB ID: 5R8M), IL6 (PDB ID: 1ALU), INS (PDB ID: 1UZ9), TNF (PDB ID: 7JRA), and TP53 (PDB ID: 2J1Y) by molecular docking. The results showed that the binding energy is between -3.17 and -6.5 Kcal/mol, which means the binding was stable and feasible. The detailed docking results of proteins and ligands are shown in **Table 4**. The three core molecules quercetin, kaempferol and Palmitic acid could combine with the top 10 target proteins. The images of the optimal docking site between ligands and proteins were shown in **Figure 7**.

Discussion

PD seriously affects the physical and mental health of elderly patients, and greatly reduces

Table 4. Molecular docking simulation of the top 3 TMGTD core active ingredients and the top 10 PD core targets

Name (PDB ID)	Protein structure	Butt ligands	Binding energy (kcal·mol-1)	Optimal binding site
ALB (4L8U)		Kaempferol	-5.27	A: LEU112: HN
		Palmitic acid	-4.88	A: LYS359: HZ2
		Quercetin	-6.15	A: GLU376: OE2
AKT1 (1UNQ)		Kaempferol	-4.8	B: THR448: O
		Palmitic acid	-4.09	B: ARG222: HH21
		Quercetin	-5.78	B: LYS163: HZ1
APP (1TKN)		Kaempferol	-6.5	A: ARG469: H12H
		Palmitic_acid	-3.83	A: LEU465: O
		Quercetin	-5.85	A: ARG499: H11H
CASP3 (3H0E)		Kaempferol	-4.77	A: ARG147: O
		Palmitic acid	-3.17	A: LYS57: HN
		Quercetin	-4.93	A: ASP90: O
GAPDH (6M61)		Kaempferol	-3.97	R: SER151: HG
		Palmitic acid	-3.59	R: ARG234: HH22
		Quercetin	-5.66	R: ASN316: HD22
IL1B (5R8M)		Kaempferol	-5.77	A: VAL41: O
		Palmitic acid	-5.33	A: LYS63: O
		Quercetin	-6.13	A: LEU62: O
IL6 (1ALU)		Kaempferol	-3.98	A: ASP26: OD2
		Palmitic acid	-3.95	A: ARG30: HH12
		Quercetin	-4.67	A: LYS27: HZ1
INS (1UZ9)		Kaempferol	-5.32	A: CYS11: HN
		Palmitic acid	-4.56	B: TYR26: HH
		Quercetin	-6.48	A: CYS11: HN
TNF (7JRA)		Kaempferol	-3.83	B: GLY116: HN
		Palmitic acid	-5.0	B: ASN106: HN
		Quercetin	-4.76	B: GLN103: HE22
TP53 (2J1Y)		Kaempferol	-5.21	C: TYR220: O
		Palmitic acid	-4.25	C: SER99: O
		Quercetin	-5.7	C: ARG110: HH21

the quality of life. At present, it is still challenging to explore a curable treatment method for PD. TCM provides a complementary treatment, in which, TMGTD has been shown to be effective in improving PD symptoms [11, 32]. However, its mechanism of action needs further study. In this study, network pharmacology approach and molecular docking were used jointly to investigate the possible mechanism of action of TMGTD.

A total of 187 unique active chemical components of TMGTD were collected via the TCMSP and ETCM databases. The drug-component-target network was constructed and the active ingredients such as quercetin, kaempferol and palmitic acid were screened out according to the degree value, BC value and CC value. Previous studies have confirmed that quercetin inhibits the expression of a-synuclein protein by controlling mitochondrial mass, reducing oxidative stress, and alleviating neuronal death [33]. Quercetin also improves the motor symptoms of PD by regulating the autophagy pathway, and reverses the oxidative and apoptotic responses of neurons [34]. Strong evidence revealed that kaemperfol can inhibit the activation of microglia and astrocytes, reduce the expression of inflammatory cytokines, and improve the behavioral deficits in PD rats [35]. Furthermore, kaempferol exerts a strong neuroprotective effect and delays the occurrence

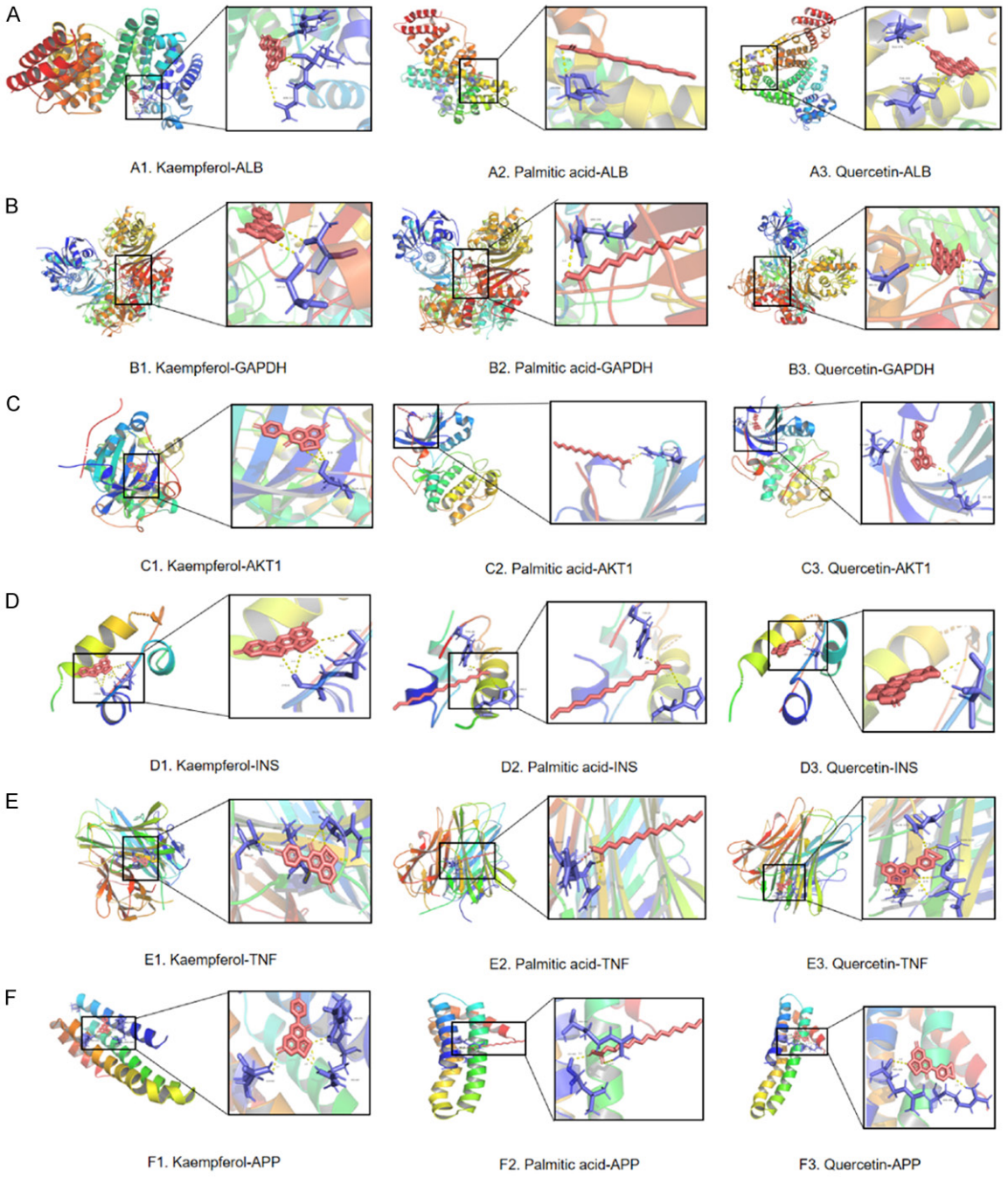


Figure 7. Visualization of molecular docking results. The top-three ingredients are Kaempferol, Palmitic acid and quercetin. A. The molecular docking between top-three ingredients and Protein structure of ALB (PDB ID: 4L8U). B. The molecular docking between top-three ingredients and Protein structure of GAPDH (PDB ID: 6M61). C. The molecular docking between top-three ingredients and Protein structure of AKT1 (PDB ID: 1UNQ). D. The molecular docking between top-three ingredients and Protein structure of INS (PDB ID: 1UZ9). E. The molecular docking between top-three ingredients and Protein structure of TNF (PDB ID: 7JRA). F. The molecular docking between top-three ingredients and Protein structure of APP (PDB ID: 1TKN).

and development of neurodegenerative diseases through scavenging free radicals while retaining the activity of antioxidant substances, and crossing the blood-brain barrier [36]. Additionally, palmitic acid stimulates PGC-1α promoter methylation in cortical neurons, microglia, and astrocytes [37], thereby protects astrocytes under inflammatory conditions, reduces

the effects of lipotoxicity on neurons as well as alleviates neurodegenerative processes [38]. The above studies showed the active effects of quercetin, kaempferol and Palmitic acid on the treatment of PD, and its mechanism is worthy of further exploration.

In this study, a total of 104 common targets of TMGTD were found and these molecules are potential targets of TMGTD in the treatment of PD. In order to identify the core targets, we constructed a PPI network, ranked according to degree value, BC value and DC value from high to low. The main therapeutic targets are ALB, GAPDH, AKT1, INS, TNF, APP, CASP3, IL6, TP53 and IL1B. Among them, ALB, GAPDH and AKT1 ranked the top three. Recent study showed that ALB is the core active target in PD [39]. The significant accumulation of GAPDH in the nuclei of neurons in PD patients plays a key role in the pathophysiology of neuronal cell death. Reducing such death signaling markers like GAPDH may attenuate neurodegeneration and help therapeutically control the development of PD [40]. Another study showed that activation of Akt1-Creb signaling pathway involved in neurodegeneration in PD tissue [41]. The AKT1 gene is a potential risk factor for PD, and it plays a protective role in the development of PD [42].

This study explored the mechanism of TMGTD in the treatment of PD through GO analysis and KEGG enrichment analysis. GO analysis showed that, BC are associated with modulation of neurotransmitter levels, neuronal death, chemical synaptic transmission and membrane potential modulation, cognitive learning and memory. CC are closely related to synaptic membranes and neuronal cell bodies. MF are mainly reflected in neurotransmitter receptor activity, G protein-coupled receptor activity, and dopamine neurotransmitter receptor activity. KEGG analysis showed that treatment of PD by TMGTD involves multiple signaling pathways including PI3K-Akt signaling pathway and MAPK signaling pathway. Activation of PI3K/AKT signaling pathway can alleviate dopaminergic neuron damage, reduce oxidative stress and inflammatory responses, and relieve PD symptoms [43, 44]. MAPK signaling pathway inhibition exerts anti-autophagy, anti-apoptotic and anti-inflammatory effects, and reduces the movement and sensory disorders of PD [45, 46].

Based on the above network pharmacology research results, the potential molecular mechanism of TMGTD in the treatment of PD was simulated and verified. The predicted results of network pharmacology in this study were validated by molecular docking. When the binding energy is less than 1.2 Kcal/mol (-5.0 KJ/mol), the molecular docking simulation binding is stable and feasible. The results showed that these compounds can stably bind to targets, providing a material basis for the TMGTD's treatment on PD.

Conclusion

Integrating network pharmacology and molecular docking technologies, this study explored the main chemical components of TMGTD in the treatment of PD and its molecular mechanism. The result shows that quercetin, kaempferol and palmitic acid may be the main active ingredients of TMGTD. Protein target genes ALB, GAPDH and AKT1 may be potential target genes for the treatment of PD, and it may play a therapeutic role through PI3K-Akt signaling pathway and MAPK signaling pathway. In general, this study provides a scientific basis and reference for further research on the potential mechanism of TMGTD in the treatment of PD.

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Disclosure of conflict of interest

None.

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