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## Mechanism of Rhizoma Coptidis in epilepsy with network pharmacology

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### Abstract

Network pharmacology is a bioinformatics-based research strategy aimed at identifying drug actions and facilitating drug discovery. In this study, network pharmacology was used for exploring the anti-epileptic multi-target mechanism of Rhizoma Coptidis. The possible protein targets of Rhizoma Coptidis were predicted by constructing the pathway and network of drug targets. Then, the interaction of the main active components of Rhizoma Coptidis and predicted candidate targets were verified using molecular docking technology. Finally, nine active compounds were selected from Rhizoma Coptidis. A total of 68 targets associated with Rhizoma Coptidis treating epilepsy. The key targets were AKT1, IL6, VEGFA, and TP53. According to GO functional enrichment analysis, 289 items of biological process, 33 items of cellular component, and 55 items of molecular function were obtained. 89 signaling pathways were identified through KEGG pathway enrichment analysis ( $P < 0.05$ ), and HIF-1, TNF, and T-cell receptor signaling pathways were mainly related to epilepsy. Molecular docking showed quercetin and (R)-canadine combined well with the key targets. The active ingredient in Rhizoma Coptidis can regulate various signaling pathways, and have therapeutic effects on epilepsy.

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### Introduction

Epilepsy is a long-term central nervous system disease that has a huge impact on the physiological, psychological, cognitive functions of patients.<sup>1,3</sup> Its abominability and incidence rate pose a serious threat to the physical and mental health

of patients and to the social development.<sup>4,5</sup> Its typical manifestation is recurrent seizures, which damage the patients' psychological, mental, and cognitive functions.<sup>6</sup> Globally, more than 70 million people are affected by epilepsy.

Currently, treatment for epilepsy is mainly oral antiepileptic drugs. Most antiepileptic drugs target neurons

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and achieve an antiepileptic effect by inhibiting glutamate energy transfer or promoting  $\gamma$ -aminobutyric acid energy transfer. The first-line treatment of epilepsy is oral antiepileptic. Its antiepileptic mechanism mostly focuses on inhibiting neural excitability and neuroelectrical activity. However, on the premise of taking antiepileptic drugs in sufficient amount and treatment, 30% of patients still have uncontrollable seizures.<sup>8</sup>

Discovering novel therapeutic targets and developing therapeutic drugs with short onset latency and few side effects is the primary task of prevention and treatment of epilepsy. Traditional Chinese medicines (TCMs) play an important role in the prevention and treatment of diseases owing to their improved safety and few side effects and thus have attracted attention worldwide.<sup>9</sup> TCMs have the following characteristics: multiple ingredients, multiple targets, and multiple ways to be effective.<sup>10</sup> As a Chinese herbal medicine, Rhizoma Coptidis is a common Chinese medicine that shows multiple biological activities, including anti-carcinogenic, neuroprotective, and anti-cancer et al.<sup>11</sup> Pharmacological research results show that Rhizoma Coptidis medicinal and health care values such as being anti-inflammatory, anti-viral, bacteriostatic, and hypoglycemic. The chemical constituents of Rhizoma Coptidis include alkaloids, lignans, coumarins, flavonoids, terpenoids, steroids, organic acids, volatile oil, and polysaccharides.<sup>12</sup> It has a good synergistic effect on epilepsy treatment.<sup>13</sup> Owing to the rapid progress of bioinformatics, systems biology, and poly-pharmacology, network-based drug discovery has become a promising approach for developing effective drugs. In 2007, Hopkins et al. proposed the concept of “network pharmacology,” which is a method for analyzing the interventions using drugs and potential treatment targets of diseases. Network pharmacology highlights a paradigm of the “network target, multi-component” strategy.<sup>14</sup>

In this study, we applied network pharmacology to clarify the mechanism of Rhizoma Coptidis in the treatment of epilepsy. We systematically analyzed the active ingredients, potential targets, pathways, and networks affected by Rhizoma Coptidis for the treatment of epilepsy. We also performed docking studies to predict the interactions that allow important compounds to bind to predicted targets. Our results may help clarify how Rhizoma Coptidis can be effective against epilepsy and facilitate the development of novel drugs.

## Materials and Methods

### Prediction of target genes associated with epilepsy

Epilepsy-related genes were searched from GeneCards (<https://www.genecards.org/>) and the National Center for Biotechnology Information database (<https://www.ncbi.nlm.nih.gov/>), and used epilepsy as the key word. After “Homo sapiens” was used as a filter term, 712 genes were screened by NCBI, and 2088 genes were screened by GeneCards. A total of 2228 genes were obtained after the deletion of repetitive genes.

### Collection of components and targets of Rhizoma Coptidis

According to the natural product databases for Chinese herbal medicines: TCMS (P (<https://tcmsp-e.com/>)),<sup>15</sup> the compounds and targets of Rhizoma Coptidis were collected. Absorption, distribution, metabolism, and excretion related models, including oral bioavailability (OB)<sup>16</sup> and drug-likeness (DL),<sup>17</sup> were used as the main variables that affect the absorption of drugs by the gastrointestinal tract. Therefore, we screened bioactive components under the standards of  $OB \geq 30\%$  and  $DL \geq 0.18$ <sup>18</sup>, and obtained the related targets of each component. By using a Venn map, we intersected genes associated with epilepsy and the common targets of Rhizoma Coptidis. The intersected genes were probably the target genes of Rhizoma Coptidis in epilepsy treatment.

### Protein-protein interaction data

The STRING database (<https://string-db.org/cgi/input.pl>)<sup>19</sup> contains known and predicted protein-protein interactions (PPIs). A large number of PPIs were collected, including 9,643,763 proteins and 1,380,838,440 interactions and data obtained from experimental detection and bioinformatics prediction. The intersected genes were imported into the STRING database. The species was defined as “Homo sapiens.” PPI data were obtained, and the results were saved in TSV format. The information of node1 and node2, and combined score in the file was retained. The interaction network was drawn, and the network was analyzed using Cytoscape.

### Network construction

The active components of Rhizoma Coptidis and intersected genes were imported into Cytoscape version 3.6.0 to construct the compound-target network of Rhizoma Coptidis.

### Gene ontology and pathway analysis

We used the Database for Annotation, Visualization, and Integrated Discovery<sup>20</sup> (DAVID, <https://david.ncifcrf.gov/>, v6.8) for gene ontology (GO) enrichment analysis, and pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes<sup>21</sup> (KEGG, <http://www.kegg.jp/>). Biological information annotation database (David, <https://david.ncifcrf.gov/>, version 6.8) provides a systematic and comprehensive annotation information on the biological functions of large-scale genes or proteins and can determine most significantly enriched biological annotation. The intersected genes were imported into the DAVID database. The select identifier was set as official gene symbol. The list type was set to gene list, and the species was limited to “Homo sapiens.” GO analysis and KEGG pathway analysis were performed based on the intersected genes.

### Molecular docking

The active components were selected as the ligand, and the targets with the top four-degree values was selected

as the receptors for molecular docking. (1) Ligand preparation: ligand molecular structure was downloaded from the TCMSP database and saved in mol2 format. (2) Preparation of receptors: the molecular structures of the receptors were downloaded from the RCSB PDB database, and the molecules with high reliability were selected and stored in PDB format. (3) Molecular docking: Autodock was used in dehydrating, hydrogenating, and computing charge, the results were saved in pdbqt format for molecular docking, and Discovery Studio 2016 was used for visualization. The selected target proteins was AKT1 (2uzs),<sup>22</sup> VEGFA (6d3o: <https://www.pdbus.org/structure/>), IL6 (1alu),<sup>23</sup> and TP53(3dcy: <https://www.pdbus.org/structure/3DCY>).

## Results

### Active compounds of Rhizoma Coptidis

TCMSP database (<http://ibts.hkbu.edu.hk/LSPtcmsp.php>) is a unique pharmacological platform of the TCM system, which can calculate ADME-related characteristics of natural compounds.<sup>15</sup> A total of 49 components of Rhizoma Coptidis were identified from TCMSP. The components were screened with the following criteria: OB  $\geq$  30% and DL  $\geq$  0.18. In this study, 14 bioactive components of Rhizoma Coptidis were screened, five of which had no targets. Finally, nine compounds were collected from the TCMSP database (Figure 1B; Table 1).

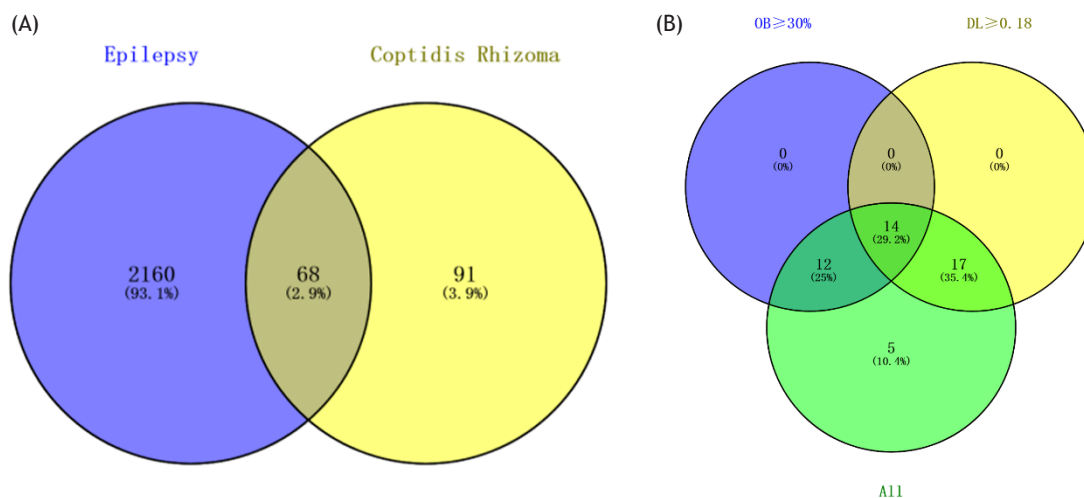
### Epilepsy-Rhizoma Coptidis PPI network

By using epilepsy as a keyword in NCBI and GeneCards database, 2228 genes related to depression were retrieved, and 159 target genes of epilepsy were collected from the TCMSP database. After the target genes

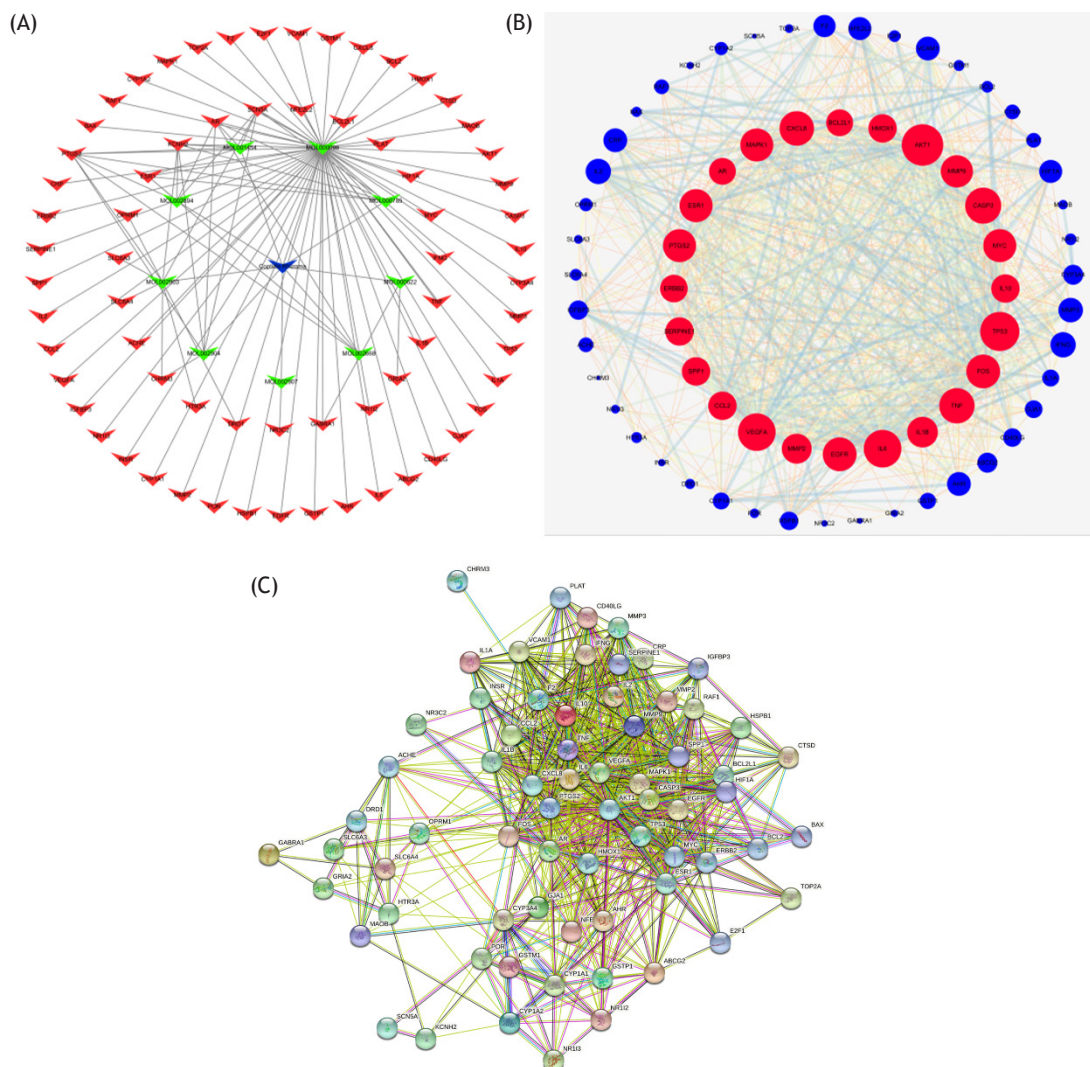
**Table 1** Basic information of active compounds in Rhizoma Coptidis.

No	Mol ID	Molecule name	MW	OB (%)	DL
1	MOL001454	berberine	336.39	36.86	0.78
2	MOL002894	berberrubine	322.36	35.74	0.73
3	MOL002903	(R)-Canadine	339.42	55.37	0.77
4	MOL002904	Berlambine	351.38	36.68	0.82
5	MOL002907	Corchoroside A Qt	404.55	104.95	0.78
6	MOL000622	Magnograndiolide	266.37	63.71	0.19
7	MOL000785	palmatine	352.44	64.6	0.65
8	MOL000098	quercetin	302.25	46.43	0.28
9	MOL002668	Worenine	334.37	45.83	0.87

of epilepsy and epilepsy were intersected, 68 common genes were found (Figure 1A). These genes can be the target genes of Rhizoma Coptidis in the treatment of epilepsy. Then, we built a visualized epilepsy-Rhizoma Coptidis-component-target network with Cytoscape 3.6. The nodes of different colors and shapes represented the potential active components and targets. The edges represented the correlation between the active components and the targets (Figure 2A) and fully reflected the multi-component and multi-target characteristics of Rhizoma Coptidis. A total of 68 nodes and 784 edges were obtained, as shown in Figure 2B, and the average node degree was 23.1. The local clustering coefficient was 0.707. The size of the node represented the degree value of the targets. The degree value increased with node size. The thickness of the edge indicated the combination score. The combination score value increased with coarseness. The original protein interaction diagram of the STRING database was shown in Figure 2C. The top 10 target proteins were shown in Figure 3A, and the degree of protein was shown in Figure 3B. The three subnetworks were shown in Figure 4.



**Figure 1** Rhizoma Coptidis screening of bioactive compounds and targets. (A) Mapping of Rhizoma Coptidis-related targets and epilepsy-related targets, 68 common targets were showed. (B) Venn diagram: 48 components (green section), and 14 bioactive components screened by two ADME-related models (blue section stands for the components of OB  $\geq$  30%, yellow section stands for DL  $\geq$  0.18).



**Figure 2** Common-target network. (A) Construction of Rhizoma Coptidis component-target visual network. Blue node stands for Rhizoma Coptidis, green nodes stand for bioactive components, and red nodes stand for predicted targets. (B) Interaction network of common target proteins. The red nodes represent the big hub nodes. The node size was proportional to the target degree in the network. (C) Protein-protein interaction mapping by string database.

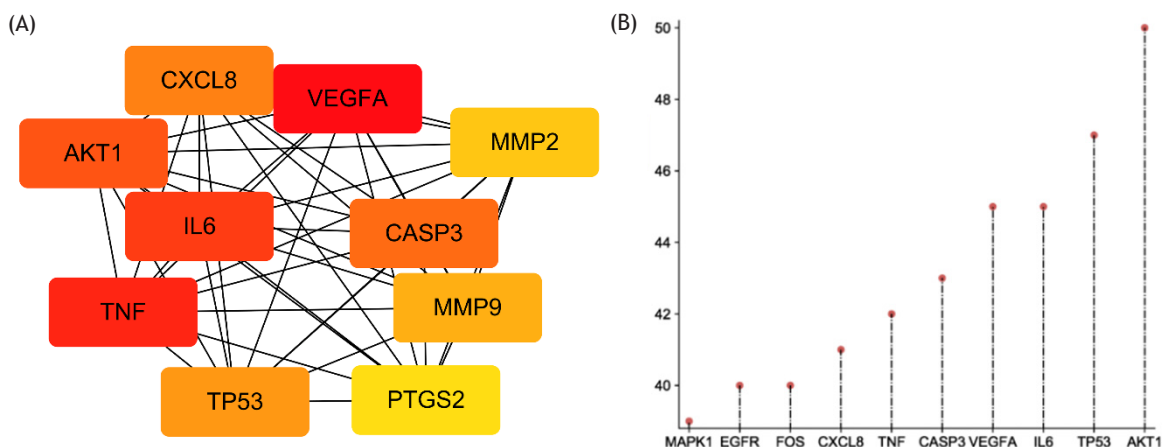
### GO analysis of genes targeted by Rhizoma Coptidis

DAVID database was applied to the analysis of the related targets of Rhizoma Coptidis in the treatment of epilepsy. In biological process analysis, the top-ranked targets were positive regulation of gene expression ( $P = 0.00000000000000434$ , 17 targets/262), positive regulation of nitric oxide biosynthetic process ( $P = 0.0000000000000743$ , 10 targets/43), positive regulation of transcription from RNA polymerase II promoter ( $P = 0.00000000000015$ , 24 targets/981), response to drug ( $P = 0.0000000000125$ , 15 targets/304) and negative regulation of apoptotic process ( $P = 0.000000000236$ , 16 targets/455; [Figure 5A](#)). In cellular component analysis ([Figure 5B](#)), extracellular space ( $P = 0.000000000000429$ , 27 targets/1347), extracellular region ( $P = 0.00000075$ , 21 targets/1610), membrane raft ( $P = 0.0000104$ , 8 targets/206), plasma membrane ( $P = 0.0000474$ , 31 targets/4121), extracellular region, and extracellular space were at the top.

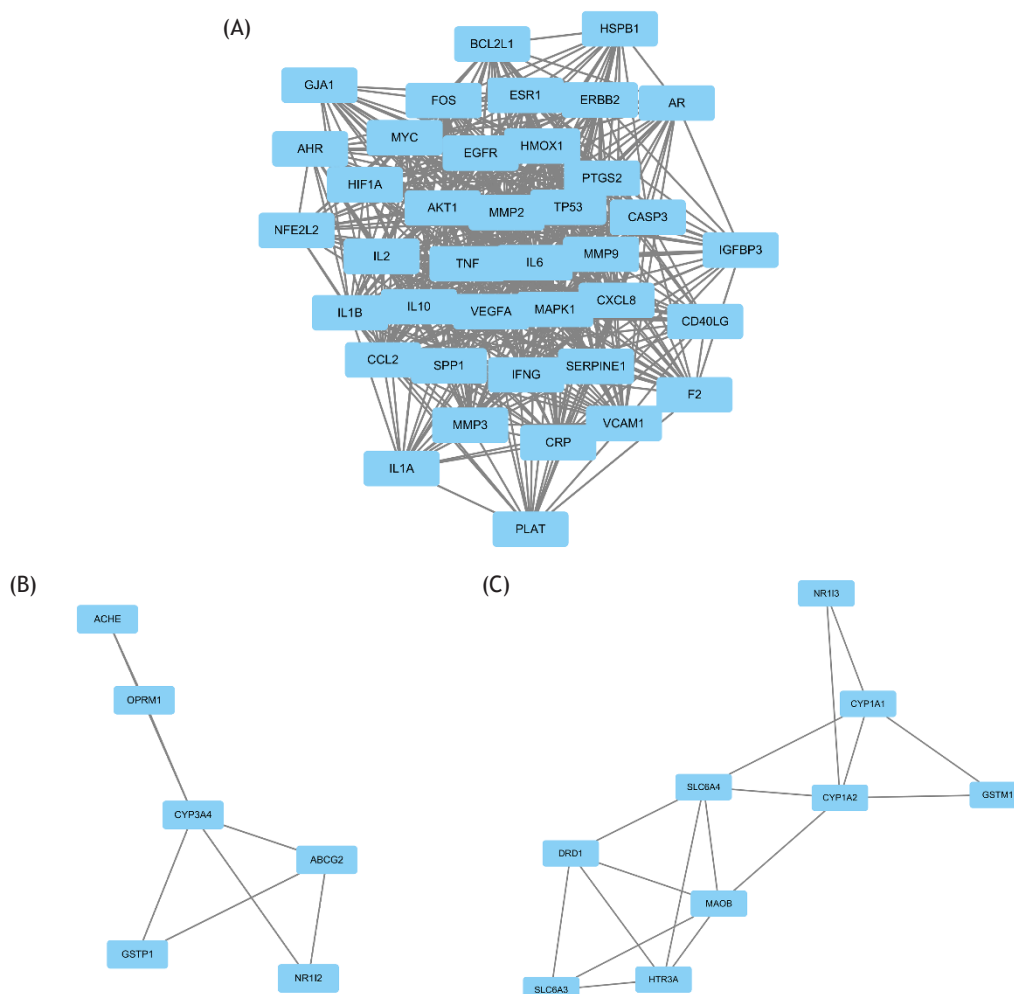
In molecular function analysis ([Figure 5C](#)), enzyme binding (16 targets/ $P = 0.00000000000266$ , 333), cytokine activity (10 targets/ $P = 0.0000000302$ , 176), protein binding (57 targets/ $P = 0.0000000617$ , 8785), identical protein binding (15 targets/ $P = 0.00000111$ , 749) were at the top.

### KEGG analysis of proteins targeted by Rhizoma Coptidis

DAVID database was used in analyzing the KEGG pathway of the 68 targets of Rhizoma Coptidis for the treatment of epilepsy. According to the P value, the top 20 pathways were selected, which are shown in [Figure 5D](#). The colors of the nodes in the figure (from green to red) reflected the P value from large to small. The nodes from small to large reflected the number of related genes in an increasing trend. The top-ranked pathways were bladder cancer ( $P = 0.00000000000106$ , 11 targets/41), pathways in cancer ( $P = 0.00000000000471$ , 22 targets/393), hepatitis B



**Figure 3** Top 10 hub targets. (A) The top 10 targets screened out. (B) Degree level of each hub-target.

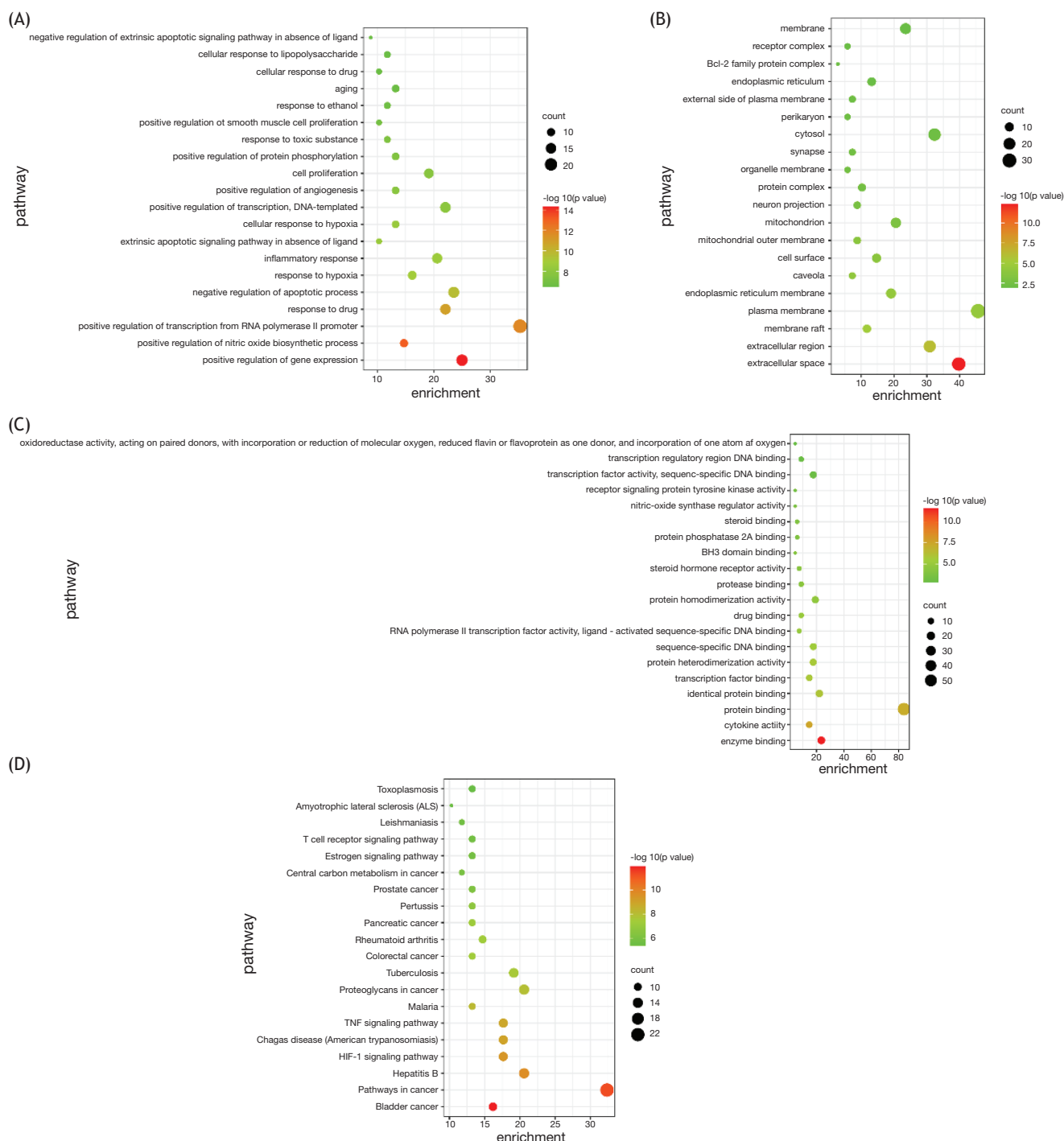


**Figure 4** Subnetwork graph of protein-protein interaction. (A) Score = 29.312. (B) Score = 3.2. (C) Score = 4.25.

( $P = 0.000000000209$ , 14 targets/145), HIF-1 signaling pathway ( $P = 0.000000000426$ , 12 targets/96), and Chagas disease ( $P = 0.00000000102$ , 12 targets/104). These results indicated that *Rhizoma Coptidis* role in the treatment of epilepsy by participating in the regulation of a variety of pathways.

### Molecular docking

Molecular docking analysis provided a visual explanation of the interaction between ligand and its potential protein targets associated with epilepsy (Figure 6-8). We found that hydrogen bonding and Pi-alkyl were the main forms of



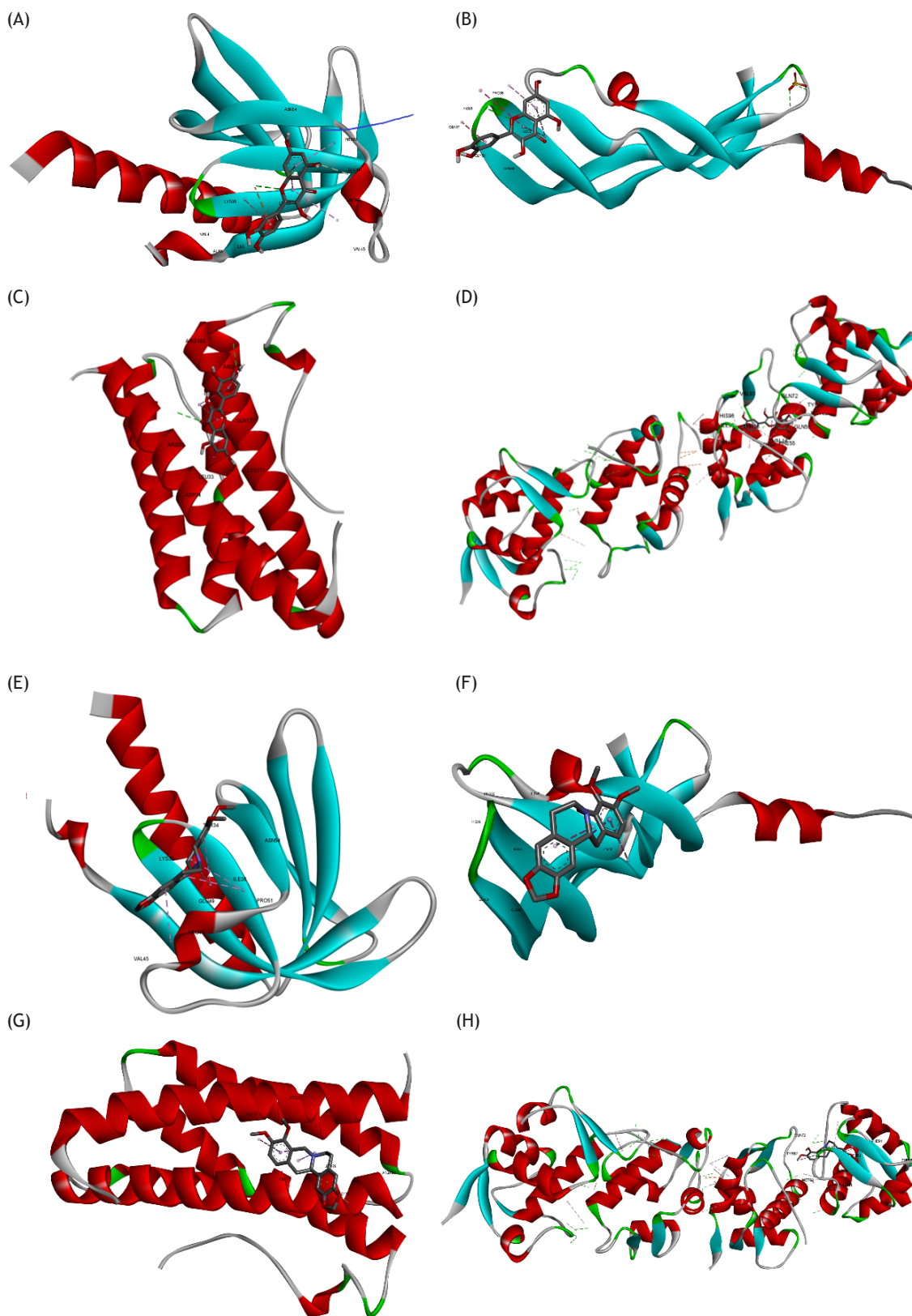
**Figure 5** Enrichment of GO and KEGG pathway of Rhizoma Coptidis in the treatment of epilepsy. (A) Enriched gene ontology terms for biological process (BP). (B) Enriched gene ontology terms for cellular component. (C) Enriched gene ontology terms for molecular function (MF). (D) Enriched KEGG pathways of potential anti-epilepsy targets from main active ingredients of Rhizoma Coptidis.

interaction. The binding energy and RMSD between ligand and receptor were shown in Tables 3 and 4.

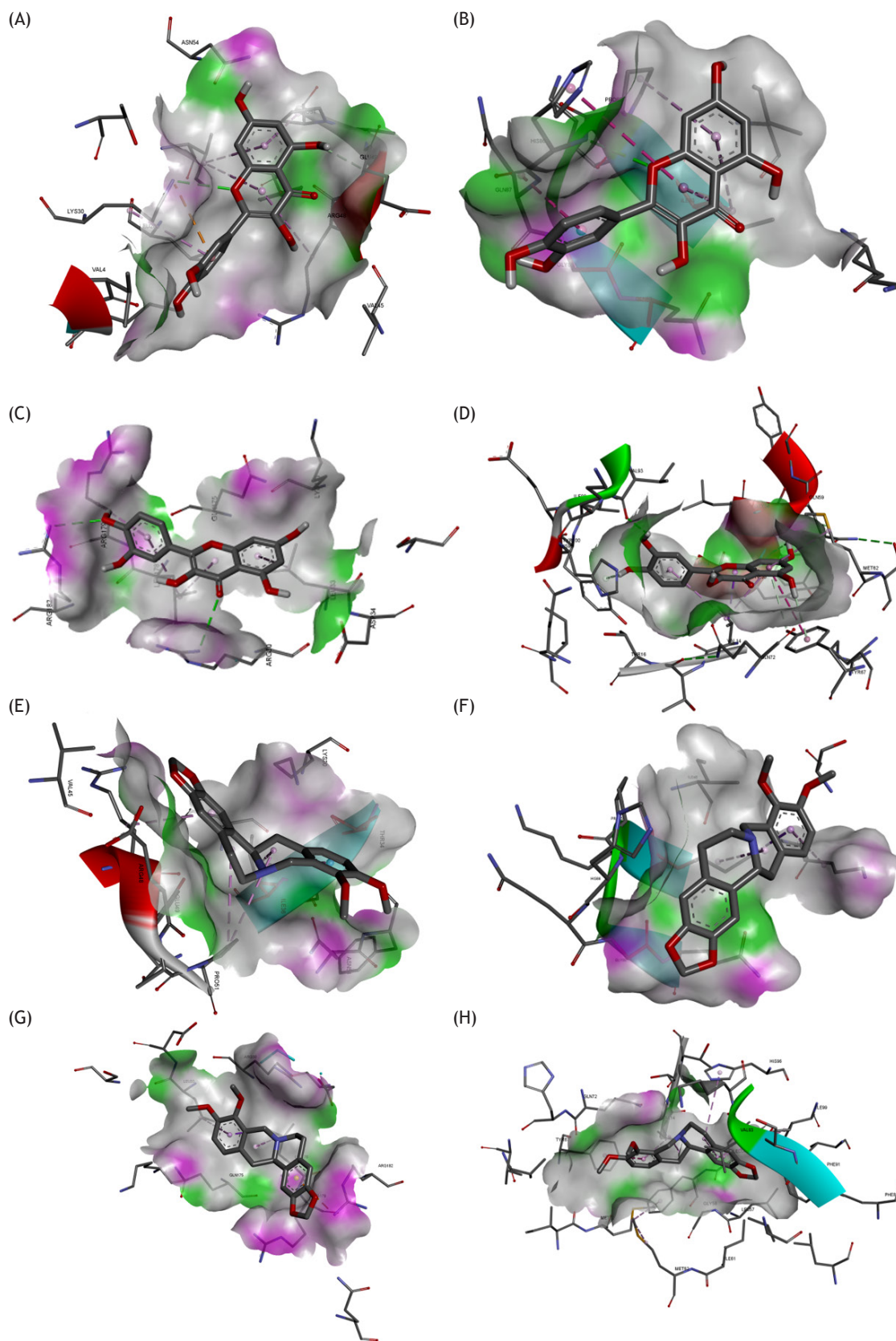
## Discussion

As a neurological disorder, epilepsy is characterized by unpredictable and repeated occurrence of seizures.<sup>24</sup> The

global prevalence of epilepsy is about 7 cases per 1000 people.<sup>25</sup> Behavioral and cognitive disorders are related to these seizures, which significantly impact patients' quality of life. Patients frequently show impaired emotional learning and spatial memory when affected by epileptic seizures. Different comorbidities, such as sleep deprivation, anxiety, depression, and disorders, may have a bearing on these changes.<sup>26</sup> Relevant relationship between epilepsy



**Figure 6** Structural model of active ingredients with hub targets. These four targets were AKT1, VEGFA, IL6, and TP53. The top two compounds were quercetin and (R)-canadine. The binding energy ranges from  $-7.8$  to  $-4.4$  kcal/mol. (A) Structural model of AKT1 with quercetin. (B) Structural model of VEGFA with quercetin. (C) Structural model of IL6 with quercetin. (D) Structural model of TP53 with quercetin. (E) Structural model of AKT1 with (R)-canadine. (F) Structural model of VEGFA with (R)-canadine. (G) Structural model of IL6 with (R)-canadine. (H) Structural model of TP53 with (R)-canadine.



**Figure 7** Binding mode of proteins and different ligands. (A) Binding mode of AKT1 with quercetin. (B) Binding mode 1 of VEGFA with quercetin. (C) Binding mode of IL6 with quercetin. (D) Binding mode of TP53 with quercetin. (E) Binding mode of AKT1 with (R)-canadine. (F) Binding mode of VEGFA with (R)-canadine. (G) Binding mode of IL6 with (R)-canadine. (H) Binding mode of TP53 with (R)-canadine.



Figure 8 Binding site of active ingredients with hub targets. (A) Binding site of AKT1 with quercetin. (B) Binding site of VEGFA with quercetin. (C) Binding site of IL6 with quercetin. (D) Binding site of TP53 with quercetin. (E) Binding site of AKT1 with (R)-canadine. (F) Binding site of VEGFA with (R)-canadine. (G) Binding site of IL6 with (R)-canadine. (H) Binding site of TP53 with (R)-canadine.

**Table 2** Information of potential anti-epilepsy targets from Rhizoma Coptidis.

Serial number	Name	Degree	Serial number	Name	Degree
1	CHRM3	1	35	CYP3A4	22
2	SCN5A	2	36	F2	25
3	GABRA1	3	37	NFE2L2	26
4	NR3C2	4	38	HIF1A	26
5	INSR	5	39	AHR	27
6	GRIA2	5	40	CRP	27
7	KCNH2	5	41	VCAM1	27
8	NR1I3	7	42	MMP3	28
9	SLC6A3	7	43	IL2	29
10	TOP2A	7	44	IFNG	29
11	MAOB	8	45	BCL2L1	31
12	POR	8	46	AR	31
13	DRD1	9	47	ERBB2	32
14	BAX	9	48	SPP1	33
15	HTR3A	10	49	HMOX1	33
16	GSTM1	10	50	CCL2	33
17	E2F1	11	51	IL10	33
18	NR1I2	11	52	SERPINE1	33
19	SLC6A4	11	53	MMP2	35
20	BCL2	11	54	IL1B	36
21	CYP1A2	13	55	MMP9	38
22	OPRM1	13	56	ESR1	39
23	ACHE	15	57	MYC	39
24	CTSD	15	58	PTGS2	39
25	RAF1	15	59	MAPK1	39
26	PLAT	15	60	EGFR	40
27	GSTP1	17	61	FOS	40
28	CYP1A1	18	62	CXCL8	41
29	IL1A	18	63	TNF	42
30	GJA1	19	64	CASP3	43
31	HSPB1	20	65	VEGFA	45
32	CD40LG	20	66	IL6	45
33	IGFBP3	21	67	TP53	47
34	ABCG2	22	68	AKT1	50

and systemic inflammation and consistent links between seizures, inflammatory cytokines (IL-6 and TNF- $\alpha$ ), and iron regulation and metabolism, as acute phase and antioxidant markers, have been reported.<sup>27</sup>

Pharmacotherapy, primarily involving the use of anti-seizure drugs (ASDs), is an essential part of controlling seizures. However, nearly 30% of patients develop drug-resistant epilepsy, clinically defined as the persistence of seizure, following trials of two ASDs.<sup>28</sup> The main research task is to develop new and effective drugs for epilepsy. Rhizoma Coptidis has good synergetic effect on the treatment of epilepsy.<sup>13</sup> However, the mechanism of Rhizoma Coptidis in the treatment of epilepsy is unclear, thus its development and utilization are limited. This study used network pharmacology method to explore the anti-epilepsy mechanism of Rhizoma Coptidis, aiming to lay a foundation for the further application of Rhizoma Coptidis.

PPI results showed interactions among 68 targets, indicating that the targets were interrelated and played a role

**Table 3** Molecular docking scores of two components of Rhizoma Coptidis and target protein (kcal/mol).

Ligand	Receptor			
	IL6	VEGFA	AKT1	TP53
Quercetin	-4.7	-4.4	-5.6	-7.8
(R)-Canadine	-4.7	-4.6	-5.3	-7.3

**Table 4** RMSD of two components of Rhizoma Coptidis and target protein.

Ligand	Receptor			
	IL6	VEGFA	AKT1	TP53
Quercetin	1.193	1.558	1.496	1.205
(R)-Canadine	1.217	1.665	1.673	3.005

in the treatment of epilepsy through multi-channel and multi-faceted coordination. The top 10 target genes were MAPK1, EGFR, FOS, CXCL8, TNF, CASP3, VEGFA, IL6, TP53, and AKT1, which were of great significance in epilepsy treatment using Rhizoma Coptidis.

The vascular endothelial growth factor (VEGF) stimulates angiogenesis and regulates vascular permeability. It plays a certain role in neuronal regeneration and cell survival. Studies have focused on the effects of the VEGF family on the electrophysiological activities of neurons. VEGF regulates the activation of NMDA receptors in neurons through Src family protein kinase signaling pathway, mainly upregulating NR2B phosphorylation.<sup>29</sup> In recent years, basic research has focused on the molecular mechanism and regulation of epilepsy cell apoptosis. The phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction pathway is involved in cell proliferation, differentiation, and apoptosis, which is related to many physiological functions of the human body. Akt is a protective protein in the central part of this signal transduction pathway. The signal transduction pathway mediated by Akt is related to cell survival and growth.<sup>30,31</sup> In the state of persistent epilepsy, the expression of AKT1 in rat hippocampus shows a dynamic process of increasing, decreasing and rising again, indicating that AKT1 may promote cell survival, neuron protection and antagonize apoptosis. TP53-induced glycolysis and apoptosis regulator (TIGAR), activates the pentose phosphate pathway (PPP).<sup>32</sup> TIGAR protects neurons against oxidative stress and apoptosis in various disorders. For example, Zhou et al. showed that TIGAR is involved in cerebral ischemia by lowering ROS levels and apoptosis.<sup>33</sup> Chen et al. showed that TP53-induced TIGAR is a promising therapeutic target for epilepsy because of its anti-epileptic, antioxidant, and anti-apoptotic effects.<sup>34</sup> The role and the involvement of IL-6 in epilepsy have not been firmly established, but some studies implicate IL-6 signaling in epilepsy.<sup>35</sup> Increased plasma levels of IL-6 have been reported in epilepsy.<sup>36,37</sup> Following status epilepticus in rats,<sup>38</sup> kindling of basolateral amygdala increased IL-6 mRNA in the rat hippocampus,<sup>39</sup> IL-6 knockout mice showed decreased incidence of seizures induced by Theiler's murine encephalomyelitis virus.<sup>40</sup>

As a sub-class of flavonoids, quercetin is abundantly found in various vegetables and fruits.<sup>41</sup> It shows inhibition of chronic inflammation, atherosclerosis and cancer in human.<sup>42</sup> For example, quercetin administration can decrease histological signs of acute inflammation in animals in a dose-dependent manner by inhibiting the release of chemokine as the lipid peroxidation end-product malondialdehyde and increasing antioxidant enzyme activity.<sup>43</sup> Quercetin also exhibits a neuro-protective function in several central nervous system disorders.<sup>44,45</sup> Recent studies have shown that quercetin inhibited KA-induced epilepsy by microglia cell inactivation and the production of NF- $\kappa$ B, TNF- $\alpha$ , and IL-1 $\beta$ .

(R)-Canadine has a variety of biological activities.<sup>46</sup> It has proved to be a new generation of dopamine (DA) antagonist in biochemistry and neuropharmacology. In addition, (R)-canadine can also increase the spontaneous discharge of DA neurons. These results not only support the conclusion that (R)-canadine is DA antagonist but also prove that one of its blocking sites is DA auto receptor (D2 receptor). Wu et al.<sup>47</sup> used nystatin perforated patch clamp method and voltage clamp whole cell recording method to study the effect of (R)-canadine on single pyramidal neurons in hippocampal CA1 area induced by DA. The holding potential (VH) was at -20 mV. DA-induced effects including transient outward current, slow inward current, and their combination. The outward current was  $-83.5 \pm 8.0$  mV, which was close to the K<sup>+</sup> equilibrium potential. It is sensitive to tetraethylamine (TEA), a K<sup>+</sup> channel blocker, suggesting that K<sup>+</sup> participates in the outward current. (R)-Canadine reversibly inhibited the three effects induced by DA.

(R)-canadine can inhibit the increase of DA-induced outward current dose-response curve, suggesting that (R)-canadine is a noncompetitive inhibition. These results confirm the hypothesis that (R)-canadine can be used as a new dopamine antagonist. In addition, (R)-canadine can inhibit DA-induced K<sup>+</sup> current in cells, indicating that (R)-canadine may have other pharmacological effects on the central nervous system. Wu et al.<sup>48</sup> continued to use whole cell patch clamp technique and found that (R)-canadine not only inhibited DA-induced outward K<sup>+</sup> current but also inhibited Ach-, caffeine-, or strychnine-induced outward K<sup>+</sup> current. Wu et al.<sup>49</sup> found that (R)-canadine can block the K-ATP channel of DA neurons in acutely isolated rat compact part of substantia nigra (SNc). In the current clamp mode, functional K-ATP channels were opened by continuous perfusion of rotenone, an inhibitor of mitochondrial respiratory chain complex I, with perforated patch clamp technique. (R)-canadine can reversibly block the opening of K-ATP channel in a dose-dependent manner. Compared with (R)-canadine analogues, (R)-canadine showed stronger blocking effect. In addition, (R)-canadine exposure alone increased the occurrence of action potential. (R)-canadine plays an important role in dopamine D2 receptor antagonist (sulpiride). In the presence of (R)-canadine, the membrane hyperpolarization induced by rotenone was restored, which indicated that (R)-canadine could excite DA neurons in SNc by blocking K-ATP channel. It was found that the expression of K-ATP in SNc DA neurons was significantly increased. The blocking of THB channel is a new mechanism of (R)-canadine, which may contribute to its neuro-protective effect in the treatment of Parkinson's disease. Xu Changqing et al.<sup>50</sup> first used 3.3  $\mu$ mol/L  $\beta$ -Amyloid peptide-induced apoptosis

of cultured hippocampal neurons in vitro to establish the model. The appearance of apoptotic nucleus or apoptotic body, the shrinkage and budding of neuronal cell body, and the state of apoptotic cells were observed with light, scanning electron, and transmission electron microscopes, and the number of apoptotic cells was counted through flow cytometry. Used 3  $\mu$ mol/L A $\beta$ , the apoptosis model of hippocampal neurons was established successfully. The effect of (R)-canadine on A $\beta$  apoptosis of cultured hippocampal neurons in vitro and the effect of free Ca<sup>2+</sup> in neurons were studied. The results showed that 1, 10, and 100  $\mu$  (R)-canadine could significantly reduce A $\beta$  with the increase of drug concentration, and the effect of reducing the number of apoptotic cells was more obvious. Compared with the model group, (R)-canadine could significantly increase the survival number of neurons in a dose-dependent manner. Li Shuyu's results showed that quercetin of 50mg/kg could increase the expression of XIAP in hippocampal neurons after status epilepticus in rats,<sup>51</sup> and quercetin has the most corresponding therapeutic targets, so it may be the most important compound of *Rhizoma Coptidis* in the treatment of epilepsy.

TCM has the characteristics of multi-component and multi-target. The effect of TCM refers to the comprehensive result that the effective molecular combination of TCM widely regulates the biological process of the body, and some active compounds in TCM act on the body through different pathways, so as to play a therapeutic effect. Therefore, through network pharmacology methods, we can get the "drug-component-target-pathway" network based on the theory of TCM, which can help comprehensively understand the mechanism of TCM in the treatment of diseases and provide data for clinical treatment. In this study, network pharmacology methods were used, and according to the results, the possible mechanism of *Rhizoma Coptidis* for epilepsy was proposed. Therefore, further clinical verification is needed, so as to more systematically and scientifically elaborate the mechanism of *Rhizoma Coptidis* in the treatment of epilepsy.<sup>52</sup>

## Conclusion

Our research may inspire and guide further work, establish the molecular target of *Rhizoma Coptidis* in the treatment of epilepsy, and apply the network pharmacology method to develop new drug. This study showed that TNF signaling pathway and AKT1, TP53, IL6, and other molecules are of great significance in the treatment of epilepsy, which must be thoroughly tested in vivo and in vitro.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

All of the data were based on public data and no additional data were available.

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