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Initial steps on the analysis of the underlying pharmacological mechanisms of Wendan decoction on sudden deafness using network pharmacology and molecular docking

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Key words: Wendan decoction, Sudden deafness, Network pharmacology, Molecular docking

Abstract: Background: Despite its widespread therapeutic use and effectiveness, the underlying pharmacologic mechanisms of Wendan decoction (WDD) and how it works to treat sudden deafness (SD) remain unclear. In this study, the pharmacological mechanisms of WDD underlying SD were analyzed using network pharmacology and molecular docking. **Methods:** The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) was employed to identify the active compounds and target genes of WDD, and genes associated with SD were screened on five databases. RGUI conducted Gene Ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. A compound-target network was established using Cytoscape, and the STRING database created a protein-protein interaction (PPI) network to identify the key compounds and targets. Subsequently, a network of crucial compound-target was generated for further molecular docking analysis. For molecular docking simulations of the macromolecular target proteins and their matching ligand molecules, AutoDock Vina and AutoDockTool were utilized. **Results:** TCMSP identified 162 active target genes and 36 active compounds for WDD. The active target genes were compared with the 2271 genes associated with SD to identify 70 intersecting active target genes linked to 34 active compounds. The GO functional enrichment and KEGG pathway enrichment analyses were undertaken, and compound–target, and PPI networks were built. The key compounds and protein targets were identified and integrated to form a key compound–target network. Eventually, molecular docking was performed to investigate the interactions of the protein targets with their respective compounds. **Conclusion:** This study highlights the mechanisms of multi-compounds, targets, and pathways of WDD acting on SD and provides further evidence of crucial compounds and their matching target proteins of WDD acting on SD.

Introduction

Sudden deafness (SD), also known as sudden sensorineural hearing loss, is a prevalent ear disease with unexplained etiology occurring suddenly within 72 h, affecting at least two adjacent frequencies and a minimum of 30 dB (Chandrasekhar *et al.*, 2019). SD affects 160 individuals per 100,000 individuals every year. While SD can occur at any age, it is more common in individuals aged 40 to 60 years, with no gender difference (Yoon *et al.*, 2022). However, the incidence of SD in China has increased significantly in recent years, particularly among younger individuals (Meng *et al.*, 2022; Tai *et al.*, 2021).

Although there is no specific treatment for SD, corticosteroid hormone therapy is the most commonly used

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treatment option. However, due to the unclear underlying pathogenesis, the response to treatment and prognosis can vary significantly among patients (Kordis *et al.*, 2020). According to traditional Chinese medicine (TCM), SD is associated with several factors, such as phlegm and fire depression, qi and blood deficiencies, qi stagnation, blood stasis, kidney essence loss, wind and heat invasion, and liver fire disturbance (Wang *et al.*, 2022). Given the limitations of Western medicine in addressing the unknown mechanism of SD, TCM is increasingly being used in the clinical management of SD (Castaneda *et al.*, 2019; Zhu *et al.*, 2022).

Wendan decoction (WDD), a conventional TCM formulation first described in *Ji Yan Fang* during the Southern and Northern Dynasties, was adopted by the National Administration of TCM in 2018 and included in the "*Ancient Classical Chinese Medicine Formula Catalogue (First Edition)*" (Wang *et al.*, 2021; Zhang *et al.*, 2022a). WDD primarily consists of *banxia (Rhizoma Pinelliae* (RP)), *chenpi (Pericarpium Citri Reticulate* (PCR)), *zhishi (Fructus Aurantii Immaturus* (FAI)), *zhuru (Caulis Bambusae in Taeniam* (CBT)*), fuling (Poria* (PA)), and *gancao (Radix Glycyrrhizae* (RG)) (Che *et al.*, 2016).

WDD is commonly used to invigorate the spleen (*Pi*), harmonize the stomach (*Wei*), regulate qi, and dispel phlegm when treating phlegm syndrome, which is intimately linked to several diseases, including digestive reflux disorders, dyslipidemia, cardiovascular disease, primary insomnia, and others diseases (Feng *et al.*, 2019; Ling *et al.*, 2015; Yan *et al.*, 2017; Zhang *et al.*, 2022b). Recent studies have demonstrated that WDD has a remarkable therapeutic impact in treating SD (Xia and Ji, 2021). For the quantification of compounds in WDD, a validated and reliable ultra-high performance liquid chromatography combined with triple quadrupole mass spectrometry (UHPLC-QQQ-MS/MS) method was developed (Zhang *et al.*, 2019). Despite its widespread therapeutic use and effectiveness, the underlying pharmacologic mechanisms of WDD and the mode of action to treat SD remains unclear.

In this study, we aimed to investigate the pharmacologic mechanisms of WDD in the treatment of SD using network pharmacology and molecular docking. We first identified the active compounds in WDD and its target genes and compared these target genes with SD-associated genes to determine the active target genes of WDD in treating SD. Next, we performed functional enrichment analyses using Gene Ontology (GO) and pathway enrichment analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG). We then constructed the protein-protein interaction (PPI) network, compound–target, and key compound–target networks and selected key compounds and protein targets. Finally, molecular docking analysis was performed to examine the binding of the protein targets to the related compounds.

Materials and Methods

Identification of active compounds in Wendan decoction

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) (Ru *et al.*, 2014) was utilized to identify the compounds present within WDD. TCMSP provides valuable information regarding the interrelation between Chinese herbal medicines, their targets, and the applicable disorders. The widely employed criteria to identify active compounds in network pharmacology are oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 (Chen *et al.*, 2022; Gao *et al.*, 2022). The OB value represents the absorption rate of the compound in the body, while the DL parameter indicates the similarity of the functional groups of the compound or physical properties with those of existing medications (Xia *et al.*, 2020; Xing *et al.*, 2021).

Active compound target genes screening

The TCMSP was also employed to obtain the matching target genes for the active compounds. The gene targets were uploaded to the UniProt Knowledgebase, a rich repository for protein sequence and annotation data (http://www.uniprot.org/) (UniProt, 2018), with the retrieval format designated as the phrase "*homo sapiens*". Subsequently, the official gene symbols for humans were determined and identified as the active target genes of WDD.

Acquisition of sudden deafness-associated genes and determination of active target genes of Wendan decoction acting on sudden deafness

Multiple databases were explored, including DrugBank (https://www.drugbank.ca/), GeneCards (https://www.genecards.org/), Therapeutic Target Database (TTD; http://db.idrblab.net/ttd/), PharmGkb platform (https://www.pharmgkb.org/), and Online Mendelian Inheritance in Man (OMIM; https://omim.org/) databases, to obtain a comprehensive collection of genes associated with SD.

The GeneCards database is a retrievable, integrated repository of annotated and predicted human genes (Stelzer *et al.*, 2016). At the same time, OMIM contains a detailed and up-to-date collection of genes and genetic traits in humans (Amberger and Hamosh, 2017). PharmGkb is a pharmacogenetic informational platform that includes clinical data such as clinical instructions and medication labels, possibly clinically actionable gene-drug connections, and genotype-phenotype interactions (Whirl-Carrillo *et al.*, 2021). On the contrary, TTD contains data on discovered and investigated nucleic acid targets and therapeutic proteins, as well as data concerning the target illness, pathway details, and the related pharmaceuticals directed at each target (Zhou *et al.*, 2022). DrugBank Online is a detailed, freely accessible online resource containing information about medications and drug candidates (Wishart *et al.*, 2018).

These genes were acquired from a pooled collection, and SD-associated genes were obtained. Subsequently, the active target genes of WDD were matched with the SD-associated genes, where the genes that overlapped were identified as the active target genes of WDD acting on SD.

Analyses of Gene Ontology functional enrichment and Kyoto Encyclopedia of Genes and Genomes pathway enrichment

The RGUI 3.6.1 was used in combination with the org.Hs.eg.db package to obtain the entrezIDs of the active target genes

identified in the previous step (Chen *et al.*, 2021b). Subsequently, RGUI, clusterProfiler package, and GOplot package were employed to perform functional enrichment analyses on GO terms, and pathway enrichment analysis on KEGG terms (Walter *et al.*, 2015; Wu *et al.*, 2021). The GO functional enrichment analysis included molecular function, cellular component, and biological process.

Compound-target network establishment

The compound–target network was created and analyzed using the Cytoscape 3.6.0 software and its NetworkAnalyzer module. Nodes denoted compounds and gene targets, while edges denoted their links. A subsequent examination of the network was carried out under the degree of connectivity between the compound and the target gene (the greater the extent of connections, the greater the degree value) (Assenov *et al.*, 2008; Shannon *et al.*, 2003).

The Establishment of the protein-protein interaction network

To create a PPI network, the active target genes were incorporated into the STRING database (https://string-db.org/), a repository that facilitates the identification of functional relationships in large-scale gene experimental datasets (Szklarczyk *et al.*, 2019). A PPI network was created by designating the study species as "*homo sapiens*" and using the least interaction value of 0.4. Subsequently, the PPI network data were employed to undertake a topology assessment using Cytoscape 3.6.0 software and its NetworkAnalyzer module. Finally, the primary target proteins of WDD acting on SD were identified based on the degree values of each protein target (the greater the extent of connections, the greater the degree value) (Assenov *et al.*, 2008; Shannon *et al.*, 2003).

Validation of molecular docking

The molecular docking technique was used to investigate the interaction of the protein target with its matching compound. The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB; http://www.rcsb.org/) was employed to acquire the target protein structures (Kouranov *et al.*, 2006), while the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was utilized to retrieve the relative compounds (Kim *et al.*, 2021). AutoDockTool 1.5.6 and AutoDock Vina software were used to conduct molecular docking simulations of protein targets with their matching compounds (Eberhardt *et al.*, 2021).

Statistical analysis

For molecular docking and network pharmacology, automatically generated statistical analyses were carried out using the bioinformatic tools provided by the software and platforms mentioned above. A q-value < 0.05 indicated statistical significance in the GO functional and KEGG pathway enrichment.

Results

Screening of active compounds in Wendan decoction

We retrieved 116 compounds from RP, 63 from PCR, 34 from PA, and 65 from FAI using the TCMSP database (The database did not have relevant information for CBT; RG as

TABLE 1

Active compounds in Wendan decoction (WDD) from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP)

Herb	Mol ID	Molecule name	OB (%)	DL
RP	MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76
RP	MOL002670	Cavidine	35.64	0.81
RP	MOL002714	Baicalein	33.52	0.21
RP	MOL002776	Baicalin	40.12	0.75
RP	MOL000358	Beta-sitosterol	36.91	0.75
RP	MOL000449	Stigmasterol	43.83	0.76
RP	MOL005030	Gondoic acid	30.70	0.20
RP	MOL000519	Coniferin	31.11	0.32
RP	MOL006936	10,13-Eicosadienoic	39.99	0.20
RP	MOL006937	12,13-Epoxy-9-hydroxynonadeca-7,10-dienoic acid	42.15	0.24
RP	MOL006957	(3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl) piperazine-2,5-quinone	46.89	0.27
RP	MOL003578	Cycloartenol	38.69	0.78
RP	MOL006967	Beta-D-ribofuranoside, xanthine-9	44.72	0.21

(Continued)

TABLE 1 (continued)

Herb	Mol ID	Molecule name	OB (%)	DL
PCR	MOL000359	Sitosterol	36.91	0.75
PCR FAI	MOL004328	Naringenin	59.29	0.21
PCR FAI	MOL005100	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27
PCR	MOL005815	Citromitin	86.90	0.51
PCR FAI	MOL005828	Nobiletin	61.67	0.52
PA	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-Dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-Octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81
PA	MOL000275	Trametenolic acid	38.71	0.80
PA	MOL000276	7,9(11)-Dehydropachymic acid	35.11	0.81
PA	MOL000279	Cerevisterol	37.96	0.77
PA	MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-Dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-Octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82
PA	MOL000282	Ergosta-7,22E-dien-3beta-ol	43.51	0.72
PA	MOL000283	Ergosterol peroxide	40.36	0.81
PA	MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-Pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.26	0.82
PA	MOL000287	3Beta-hydroxy-24-methylene-8-lanostene-21-oic acid	38.70	0.81
PA	MOL000289	Pachymic acid	33.63	0.81
PA	MOL000290	Poricoic acid A	30.61	0.76
PA	MOL000291	Poricoic acid B	30.52	0.75
PA	MOL000292	Poricoic acid C	38.15	0.75
PA	MOL000296	Hederagenin	36.91	0.75
PA	MOL000300	Dehydroeburicoic acid	44.17	0.83
FAI	MOL013276	Poncirin	36.55	0.74
FAI	MOL013277	Isosinensetin	51.15	0.44
FAI	MOL013279	5,7,4'-Trimethylapigenin	39.83	0.30
FAI	MOL013428	Isosakuranetin-7-rutinoside	41.24	0.72
FAI	MOL013430	Prangenin	43.60	0.29
FAI	MOL013433	Prangenin hydrate	72.63	0.29
FAI	MOL013435	Poncimarin	63.62	0.35
FAI	MOL013436	Isoponcimarin	63.28	0.31
FAI	MOL013437	6-Methoxy aurapten	31.24	0.30
FAI	MOL013440	Citrusin B	40.80	0.71
FAI	MOL001798	Neohesperidin_qt	71.17	0.27
FAI	MOL001803	Sinensetin	50.56	0.45
FAI	MOL001941	Ammidin	34.55	0.22
FAI	MOL013352	Obacunone	43.29	0.77
FAI	MOL002914	Eriodyctiol (flavanone)	41.35	0.24

(Continued)

TABLE 1 (continued)

Herb	Mol ID	Molecule name	OB (%)	DL
FAI	MOL005849	Didymin	38.55	0.24
FAI	MOL000006	Luteolin	36.16	0.25
FAI	MOL007879	Tetramethoxyluteolin	43.68	0.37
FAI	MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-Enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2-yl]-2-methoxy-phenol	50.76	0.39

Notes: WDD: Wendan decoction; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB: oral bioavailability; DL: drug-likeness; RP: *Rhizoma Pinelliae*; PCR: *Pericarpium Citri Reticulatae*; FAI: *Fructus Aurantii Immaturus*; PA: *Poria*.

a guide herb was not included, Suppl. Table S1). We identified 13 active compounds from RP, 5 from PCR, 15 from PA, and 22 from FAI after the screening conditions of DL ≥ 0.18 and OB ≥ 30%. Finally, after removing duplicates from the samples, we found 52 active compounds in WDD. Table 1 summarizes the basic details of the active compounds in WDD.

Identification of active target genes of Wendan decoction

The TCMSP was also used to identify the target genes for the 52 active compounds in WDD, of which 13 had no corresponding target genes (Suppl. Table S2). The matching gene symbols were further filtered using the "*homo sapiens*" format of UniProt Knowledgebase, resulting in three compounds that did not have matching gene symbols (Suppl. Table S3). Ultimately, we identified 162 active target genes for the 36 active compounds in WDD (Suppl. Table S4).

Acquisition of sudden deafness-related genes

We retrieved 2189 associated genes in the GeneCards database, 152 in the OMIM database, 7 in the PharmGkb platform, one in the TTD, and one in the DrugBank database using "sudden deafness" as the keyword (Suppl. Table S5). After removing duplicates, we considered a pooled set of these genes and obtained 2271 SD-related genes (Fig. 1).

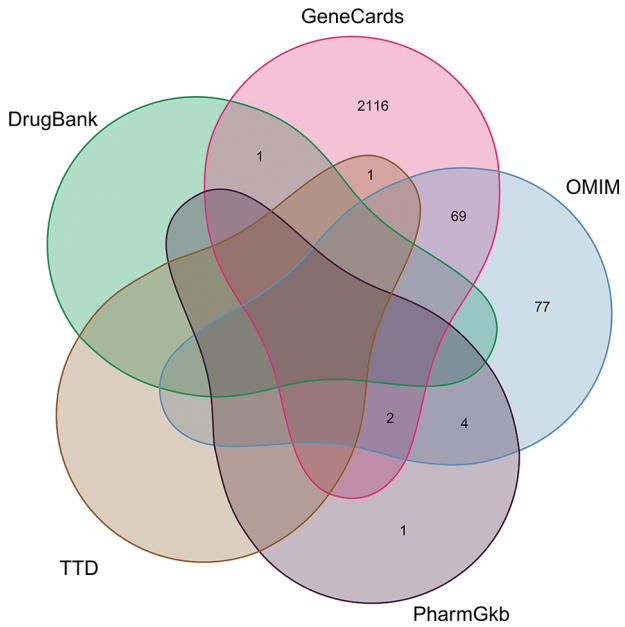


FIGURE 1. Genes related to sudden deafness (SD). A total of 2189 SD-related genes were retrieved in the GeneCards database, 152 in the Online Mendelian Inheritance in Man (OMIM) database, 7 in the PharmGkb platform, 1 in the Therapeutic Target Database (TTD), and 1 in the DrugBank database.

Identification of active genes targets of Wendan decoction acting on sudden deafness

We uncovered 70 active WDD target genes that acted on SD after comparing the 162 active WDD target genes to the 2271 genes associated with SD (Fig. 2, Table 2).

Analyses of Gene Ontology functional enrichment and Kyoto Encyclopedia of Genes and Genomes pathway enrichment

We employed RGUI and org.Hs.eg.db to retrieve the entrezIDs of the WDD active target genes that acted on SD (Table 2). Then, we conducted GO functional and KEGG pathway enrichment studies utilizing RGUI and clusterProfiler. Finally, RGUI and GOplot displayed the terms with their enriched genes on GO and KEGG chord plots.

The GO biological process enrichment analysis revealed that the WDD active target genes acting on SD were strongly enriched in processes such as sensitivity to toxic substances, responsiveness to oxidative stress, reaction to extracellular stimuli, response to metal ions, and response to nutrient levels. The analytical findings from GO cellular component enrichment illustrated a substantial enrichment in the membrane region, caveola, membrane microdomain, vesicle

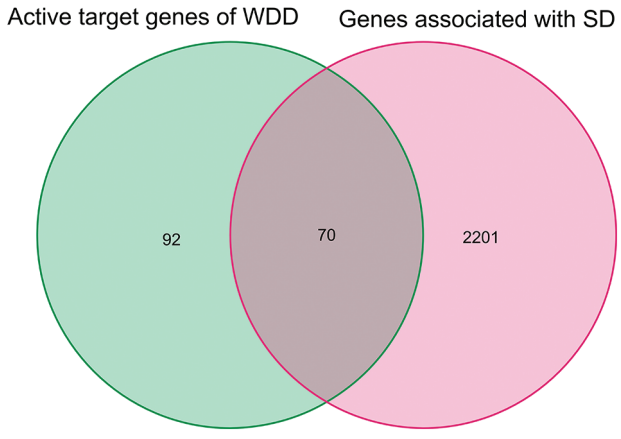


FIGURE 2. Active target genes of Wendan decoction (WDD) that influence sudden deafness (SD). The 162 WDD active target genes were matched to the 2271 genes associated with SD, identifying 70 WDD active target genes that acted on SD.

TABLE 2

Active target genes of Wendan decoction (WDD) acting on sudden deafness (SD)

Gene symbol	EntrezID	Gene symbol	EntrezID	Gene symbol	EntrezID
NR3C2	4306	*JUN*	3725	*NOS2*	4843
PTGS1	5742	*PRKCA*	5578	*GSK3B*	2932
KCNH2	3757	*MAP2*	4133	*MAPK8*	5599
SCN5A	6331	*SLC6A2*	6530	*CREB1*	1385
PTGS2	5743	*SLC6A3*	6531	*PLA2G4A*	5321
HTR3A	3359	*MAOB*	4129	*CD163*	9332
OPRM1	4988	*MAOA*	4128	*ABCC8*	6833
DRD1	1812	*ESR1*	2099	*ACHE*	43
SLC6A4	6532	*PPARG*	5468	*PYGM*	5837
AR	367	*CA2*	760	*EGFR*	1956
PRSS1	5644	*PNP*	4860	*CCND1*	595
AKT1	207	*MAPK3*	5595	*CDKN1A*	1026
VEGFA	7422	*MAPK1*	5594	*MMP2*	4313
MMP9	4318	*SOD1*	6647	*RB1*	5925
CASP3	836	*CAT*	847	*TOP1*	7150
TP53	7157	*APOB*	338	*MDM2*	4193
HIF1A	3091	*CYP19A1*	1588	*APP*	351
FOSL1	8061	*GSTP1*	2950	*MMP1*	4312
CDK1	983	*UGT1A1*	54658	*ERBB2*	2064
MPO	4353	*PPARA*	5465	*HMOX1*	3162
IGF2	3481	*SREBF1*	6720	*TYR*	7299
CYCS	54205	*GSR*	2936	*CD40LG*	959
CHRNA2	1135	*ABCC1*	4363	*MET*	4233
GABRA1	2554				

lumen, membrane raft, and other components. Further, the GO molecular function enrichment analysis illustrated a significant enrichment in several functions, including ubiquitin-protein ligase binding, tetrapyrrole binding, heme binding, protein phosphatase binding, and phosphatase binding (Suppl. Table S6). Fig. 3 depicts the top 10 GO functional enrichment terms sorted by q-value. The top 10 GO biological function enrichments with enriched genes are displayed as GO chord plots (Fig. 4).

The analytical findings recorded from the KEGG pathway enrichment illustrated a significant enrichment of the WDD active target genes acting on SD in proteoglycans in cancer, Kaposi sarcoma-associated herpesvirus infection, prostate cancer, endocrine resistance, and bladder cancer (Suppl. Table S7). Fig. 5 depicts the top 20 enriched KEGG pathways sorted by q-value. The top 10 KEGG pathway enrichments with their enriched genes are represented as a KEGG chord plot (Fig. 6).

Establishment of a compound–target network

We next employed NetworkAnalyzer to evaluate the network of compounds and targets constructed using the Cytoscape software. However, two compounds (MOL000282 and MOL000283) did not exhibit matching overlapped target genes; therefore, 70 intersecting active target genes were linked to 34 active compounds. The resulting network contained 198 edges and 104 nodes (34 compounds and 70 target genes) (Suppl. Table S8, Fig. 7).

Using NetworkAnalyzer, the top 10 compounds in the network were identified based on their degree value within the network. These key compounds may be important for the action of WDD on SD. Table 3 presents the basic details of these compounds, including their two-dimensional structure derived from the PubChem database, sorted by the degree value.

Generation of the protein-protein interaction network

A PPI network was constructed by mapping the 70 matching target genes into the STRING database. When the minimum interaction value was adjusted to 0.40, the target proteins were observed to interact within the network, while 697 edges denoted the interconnections among the proteins (Suppl. Table S9, Fig. 8).

The top 10 target proteins sorted using Cytoscape software and the NetworkAnalyzer module based on their degree value in the PPI network are displayed in Table 4;

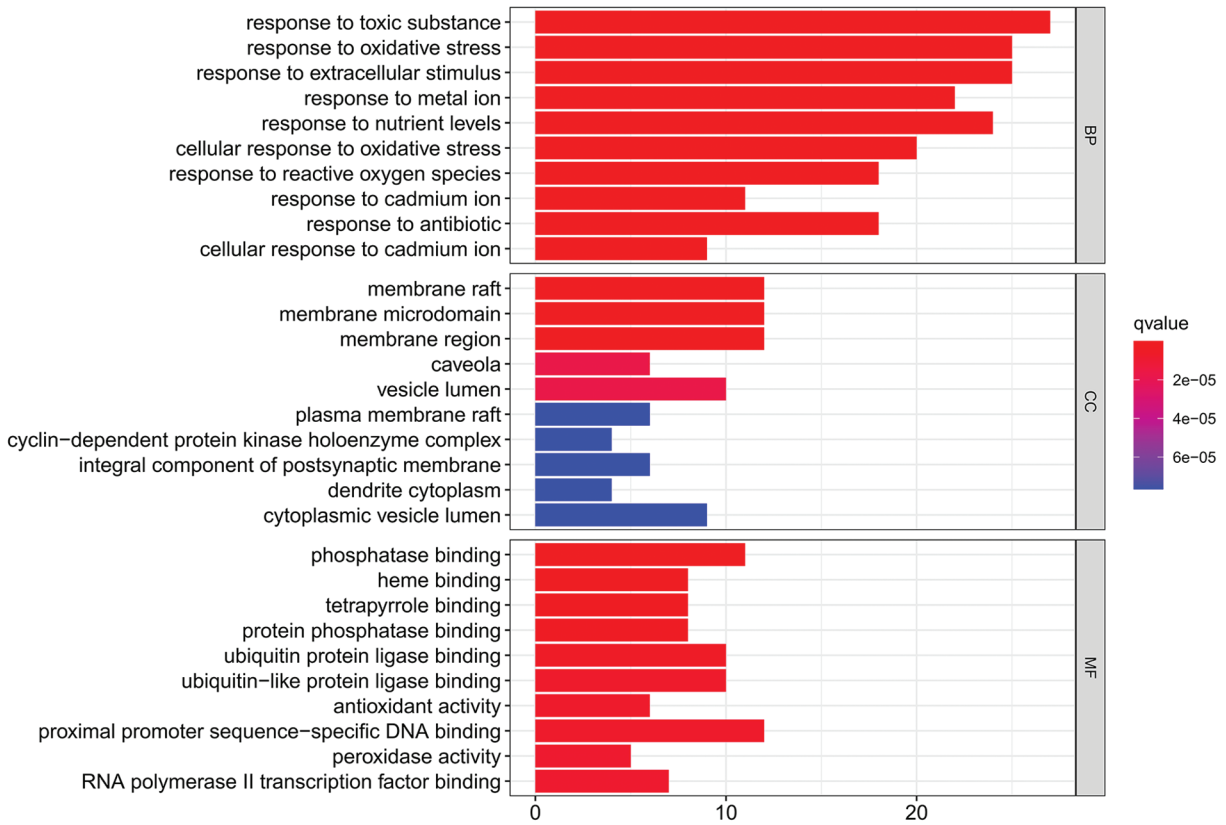


FIGURE 3. Analysis of Gene Ontology (GO) functional enrichments. The top 10 GO functional enrichments included biological process (BP), cellular component (CC), and molecular function (MF). The smaller the q-value, the greater the significance of the enrichment.

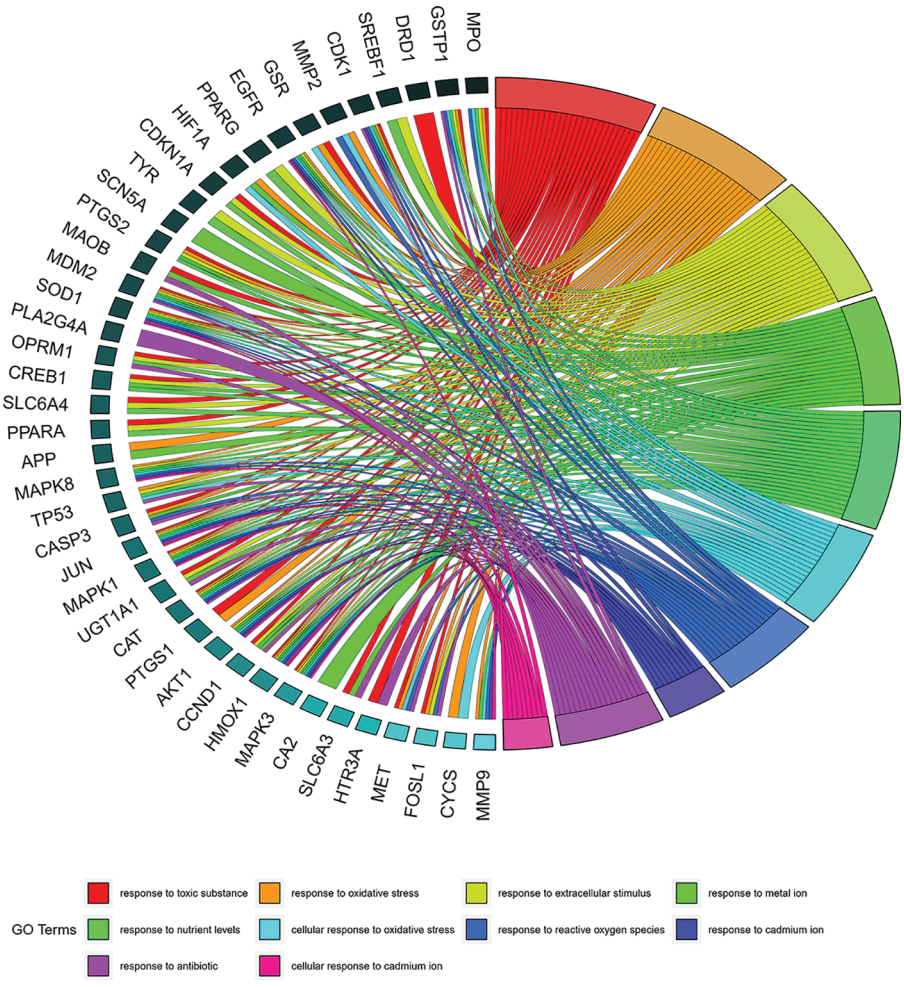


FIGURE 4. Chord plot of Gene Ontology (GO) biological process (BP). The GO terms represent the top 10 GO BP functional enrichments, and gene names with the connection represent their enriched genes.

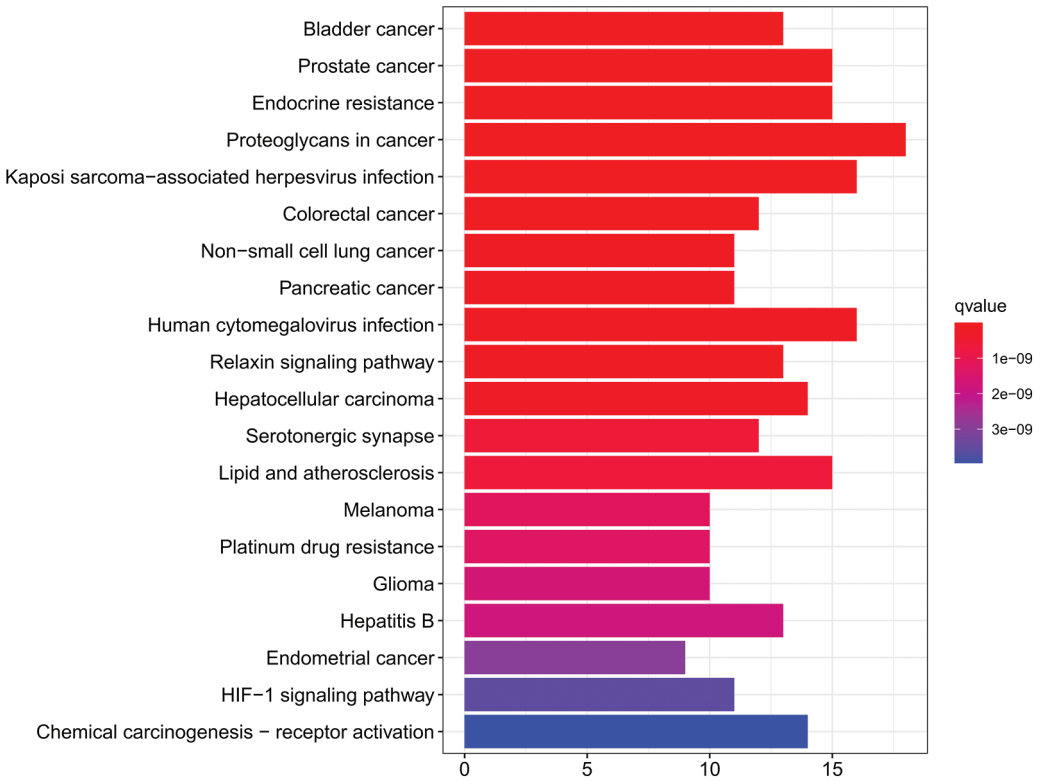


FIGURE 5. Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichments. The top 20 KEGG pathway enrichments. The smaller the q-value, the greater the significance of the enrichment.

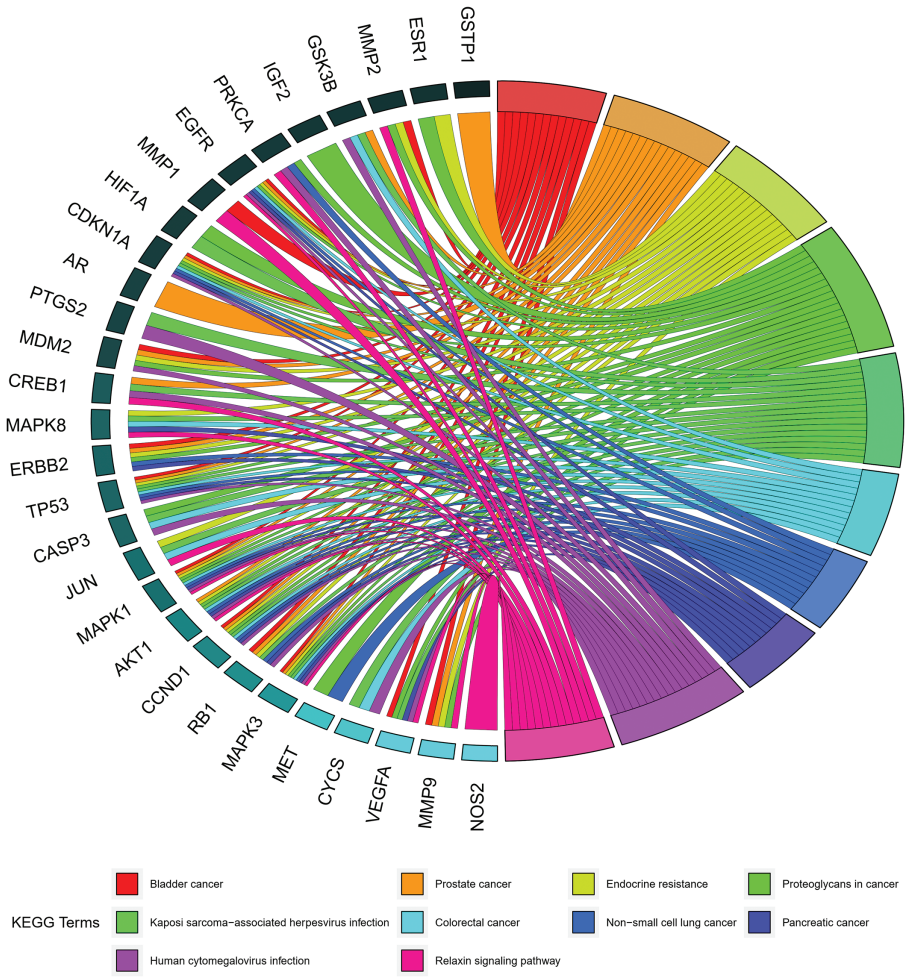


FIGURE 6. Chord plot of Kyoto Encyclopedia of Genes and Genomes (KEGG). The KEGG terms represent the top 10 KEGG pathway enrichments and gene names, with the connection representing their enriched genes.

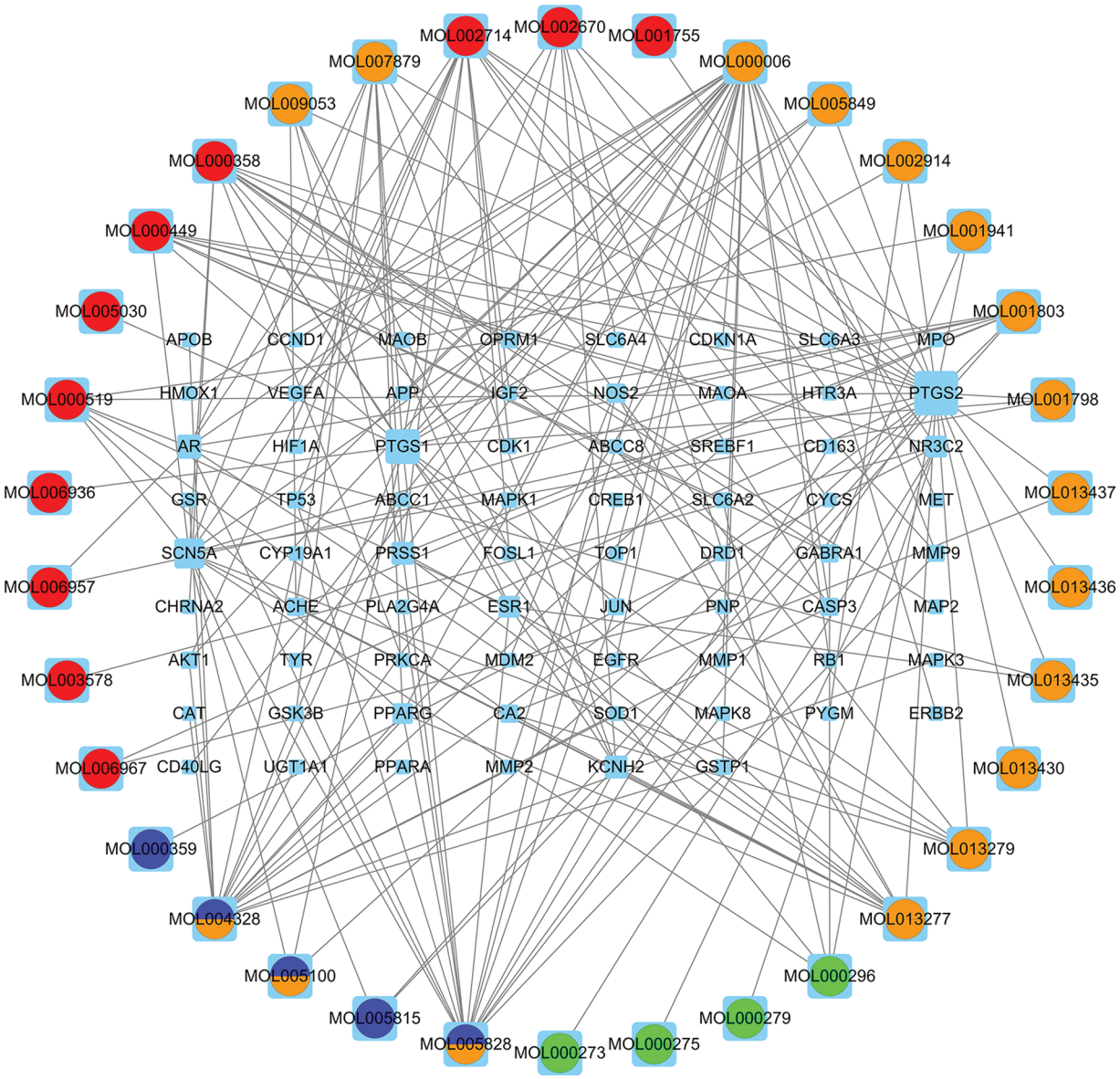
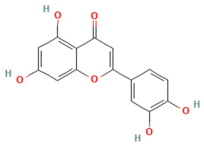
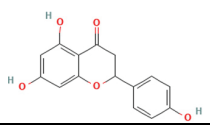


FIGURE 7. The compound-target network. The compound–target network containing 198 edges and 104 nodes (70 target genes and 34 compounds). Active compounds are shown by circles (different colors depict distinct compound types), the rectangles indicate active target genes, and the edges denote the links between the nodes. The increase in degree value is directly related to the extent of interconnections between the compounds and target genes.

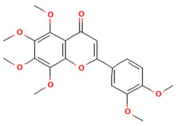
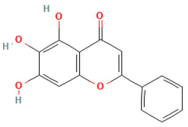
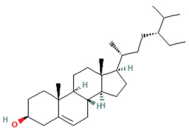
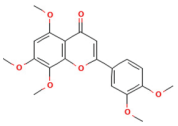
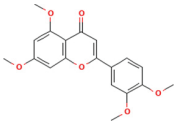
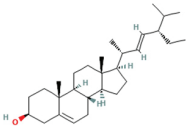
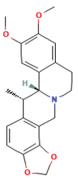
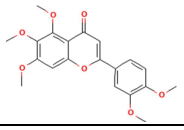
TABLE 3

Key compounds in Wendan decoction (WDD) acting on sudden deafness (SD)

Compound name	Compound ID	PubChem CID	Molecular formula	2D structure (from PubChem)	Degree	Herb
Luteolin	MOL000006	5280445	$C_{15}H_{10}O_6$		27	FAI
Naringenin	MOL004328	932	$C_{15}H_{12}O_5$		18	PCR FAI

(Continued)

TABLE 3 (continued)

Compound name	Compound ID	PubChem CID	Molecular formula	2D structure (from PubChem)	Degree	Herb
Nobiletin	MOL005828	72344	$C_{21}H_{22}O_8$		17	PCR FAI
Baicalein	MOL002714	5281605	$C_{15}H_{10}O_5$		15	RP
Beta-sitosterol	MOL000358	222284	$C_{29}H_{50}O$		13	RP
Isosinensetin	MOL013277	632135	$C_{20}H_{20}O_7$		12	FAI
Tetramethoxyluteolin	MOL007879	631170	$C_{19}H_{18}O_6$		10	FAI
Stigmasterol	MOL000449	5280794	$C_{29}H_{48}O$		9	RP
Cavidine	MOL002670	193148	$C_{21}H_{23}NO_4$		8	RP
Sinensetin	MOL001803	145659	$C_{20}H_{20}O_7$		8	FAI

Note: WDD: Wendan decoction; SD: sudden deafness; FAI: *Fructus Aurantii Immaturus*; PCR: *Pericarpium Citri Reticulatae*; RP: *Rhizoma Pinelliae*.

these might be considered the key target proteins of WDD acting on SD.

Establishment of key compound-target network

The key compound-target network was established by uploading the key compounds (small-molecular compounds) and targets (macromolecular target protein receptors) along with their associations into the Cytoscape software, which was further used for molecular docking analysis (Suppl. Table S10, Fig. 9).

Molecular docking analysis

The PubChem and RCSB PDB databases were employed to obtain the three-dimensional structures of the compounds and the target proteins, respectively. AutoDock Vina and AutoDockTool software performed docking simulations of the target proteins and their associated compounds. Using the molecular docking technique, we then investigated the interaction between the target proteins with their respective compounds. Finally, we selected the primary proteins and compounds as examples based on the key compound–target network.

The molecular docking simulations of prostaglandin-endoperoxide synthase 2 (PTGS2)-luteolin revealed grid center values of 22.594, 40.999, and 39.56, a minimum affinity of −9.8 kcal/mol, and a distance from the best mode of 0.000 rmsd u. b and 0.000 rmsd l. b. The results of molecular docking simulations of PTGS2-luteolin are displayed in Fig. 10.

The molecular docking simulations of caspase 3 (CASP3)-baicalein demonstrated grid center values of 27.82, 102.814, and 10.952, a minimum affinity of −8.0 kcal/mol, and a distance from the best mode of 0.000 rmsd u. b and 0.000 rmsd l. b. The results of molecular docking simulations of CASP3-baicalein are summarized in Fig. 11.

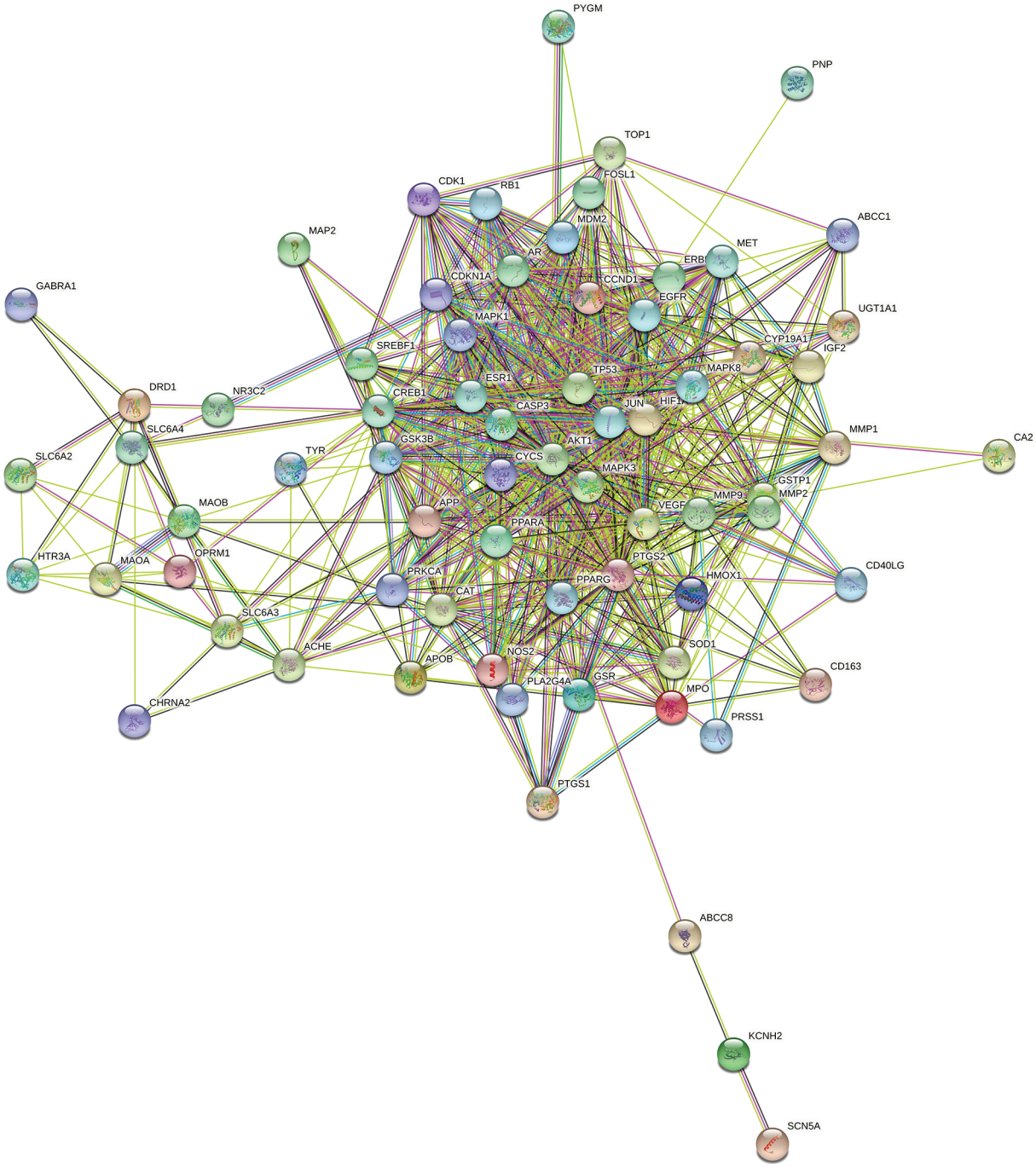


FIGURE 8. The protein-protein interaction (PPI) network. When the minimum interaction value was adjusted to 0.40, the network exhibited 70 interacting target proteins, resulting in 697 edges, which represents the interconnections among the proteins. The increase in the degree value is directly related to the extent of connections.

TABLE 4

Wendan decoction (WDD) key protein targets that act on sudden deafness (SD)

Key target	Entry	Entry name	Protein names (in UniProt)	Degree
AKT1	P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein kinase	49
CASP3	P42574	CASP3_HUMAN	Caspase-3	45
TP53	P04637	P53_HUMAN	Cellular tumor antigen p53	44
JUN	P05412	JUN_HUMAN	Transcription factor Jun	44
PTGS2	P35354	PGH2_HUMAN	Prostaglandin G/H synthase 2	41

(Continued)

Table 4 (continued)

Key target	Entry	Entry name	Protein names (in UniProt)	Degree
VEGFA	P15692	VEGFA_HUMAN	Vascular endothelial growth factor A	40
MAPK3	P27361	MK03_HUMAN	Mitogen-activated protein kinase 3	38
ESR1	P03372	ESR1_HUMAN	Estrogen receptor	38
HIF1A	Q16665	HIF1A_HUMAN	Hypoxia-inducible factor 1-alpha	38
EGFR	P00533	EGFR_HUMAN	Epidermal growth factor receptor	37

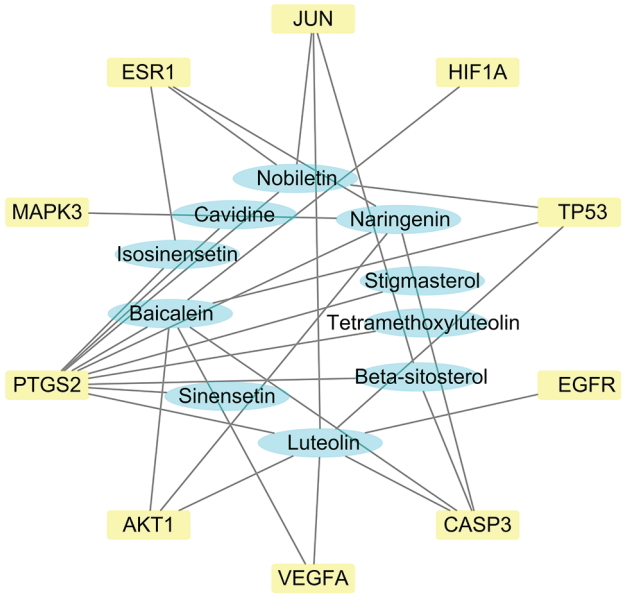


FIGURE 9. The key compound-target network. The network comprised 31 edges and 120 nodes (110 target genes and 10 compounds). The degree value increases directly to the extent of interconnections between the compounds and target genes.

Discussion

The TCM theory suggests that the etiology of SD is associated with various factors, including the lack of qi and blood, kidney essence deficit, disturbance in the liver, phlegm fire, and blood stasis. Thus, TCM theory implies that treating patients with SD relies on modulating qi and dispelling phlegm (Wang *et al.*, 2022; Zhu *et al.*, 2022).

Among the components of WDD (RP, PCR, FAI, CBT, PA, and RG), RP is a monarch (*Jun*) herb considered the primary constituent of WDD, PCR, FAI, and CBT are minister (*Chen*) herbs, PA is an assistant (*Zuo*) herb, and RG is a guide (*Shi*) herb (Wang *et al.*, 2021; Zhang *et al.*, 2022a). Studies indicate that WDD can regulate qi and dispel phlegm and is extensively used in treating phlegm syndrome, strongly linked to digestive reflux disorders, dyslipidemia, cardiovascular disease, primary insomnia, and other diseases (Feng *et al.*, 2019; Ling *et al.*, 2015; Yan *et al.*, 2017; Zhang *et al.*, 2022b).

Clinical investigations have demonstrated that the use of WDD in treating SD can enhance clinical outcomes (Xia and Ji, 2021). However, a more comprehensive understanding of the pharmacological mechanisms underlying WDD's therapeutic effect on SD is warranted. Network pharmacology is a useful, innovative tool in TCM research that could offer an method for transforming TCM from an experience-based medicine to an evidence-based medical field for systematic investigation of medicinal herbs and the discovery of pharmacodynamic compounds (Gu and Pei, 2017; Li and Zhang, 2013). Network pharmacology proposes a multi-component treatment strategy consistent with the multi-pathway, multi-target, and multi-component aspects of TCM (Huang *et al.*, 2018; Luo *et al.*, 2020). Thus, we adopted a network pharmacological method to examine the pharmacologic molecular mechanisms of WDD in the treatment of SD.

We could identify 70 active target genes of WDD that act on SD. Based on the functional enrichment of the GO biological process, the WDD active target genes that act on SD exhibited a significant enrichment in processes such as sensitivity to toxic substances, responsiveness to oxidative stress, reaction to extracellular stimuli, sensitivity to metal ions, and responsiveness to the levels of the nutrient. The functional enrichment of the GO cellular component illustrated a significant enrichment in the membrane region, caveola, membrane microdomain, vesicle lumen, and membrane raft, among other components. Moreover, functional enrichment of GO molecular function illustrated a significant enrichment in ubiquitin-protein ligase binding, tetrapyrrole binding, heme binding, phosphatase binding, protein phosphatase binding, and so on. These functions are strongly linked to the etiopathogenesis of SD, primarily characterized by inflammation and immunoregulation (Chen *et al.*, 2019, 2021a; Kum *et al.*, 2015; Li *et al.*, 2021).

The KEGG pathway enrichment analysis revealed a strong association between the pathophysiology of SD and several pathways, with the predominant pathways being the relaxin signaling pathway, non-small cell lung cancer, human cytomegalovirus (HCMV) infection, pancreatic cancer, Kaposi sarcoma-associated herpesvirus infection, colorectal cancer, proteoglycans in cancer, endocrine resistance, prostate cancer, and bladder cancer. Several of these pathways have been shown to be intimately associated with SD, indicating multifaceted mechanisms of WDD in treating SD. For instance, the occurrence of SD is associated with cancer-related genes (Nam *et al.*, 2011; Rakusic *et al.*, 2015), the extracellular matrix performs an integral function

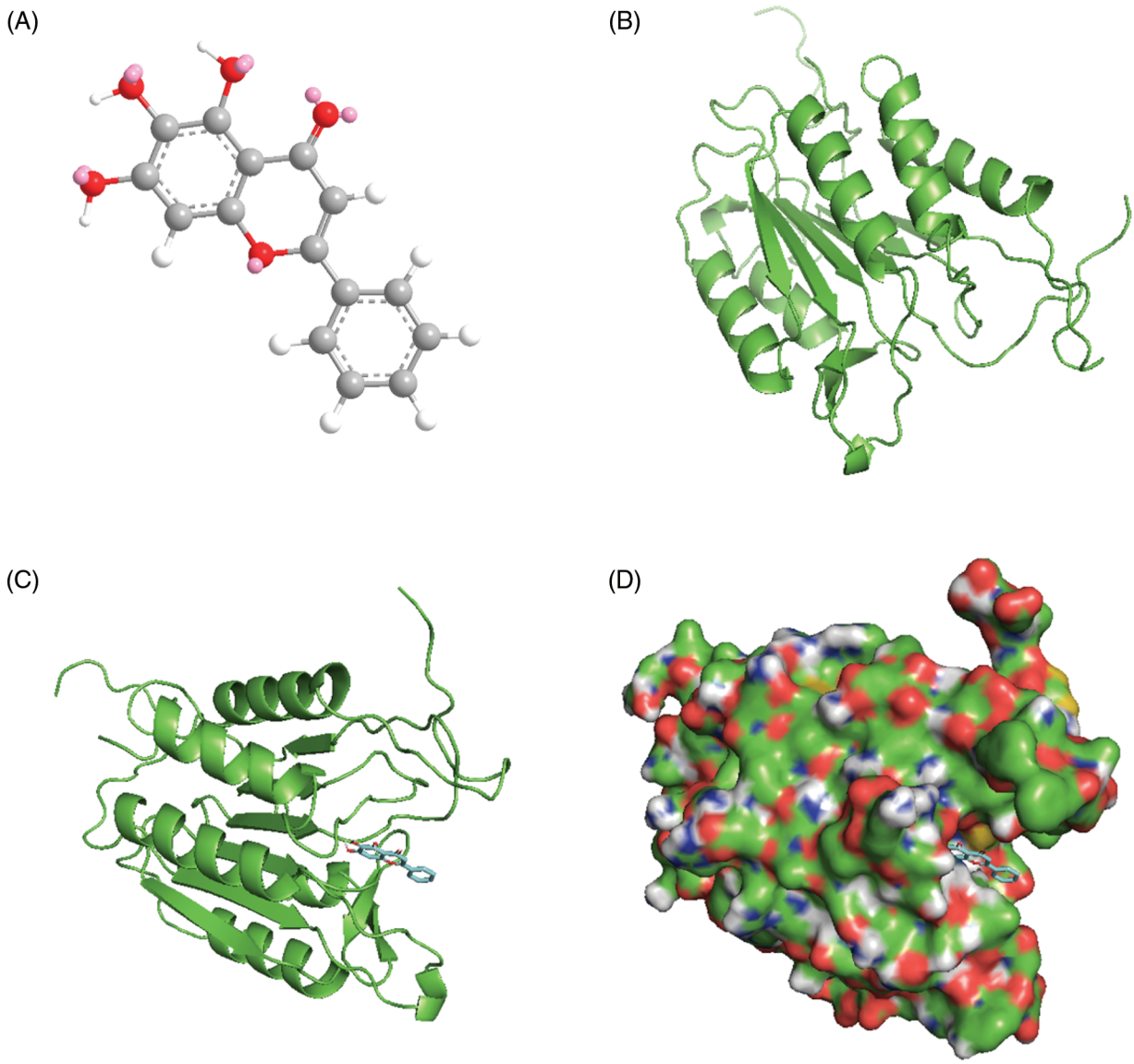


FIGURE 10. Molecular docking of prostaglandin-endoperoxide synthase 2 (PTGS2) and luteolin. (A) Three-dimensional structures of luteolin were obtained from the PubChem database. (B) Three-dimensional structures of PTGS2 were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) database. (C) Molecular docking simulation. (D) The surface of the protein is shown in a molecular docking simulation.

in the adaptive responses to SD on multiple levels along the central auditory pathway (Heusinger *et al.*, 2019), and HCMV infection has been associated with SD and acute labyrinthitis (Strauss, 1990). These findings suggest that WDD could effectively alleviate SD via various mechanisms involving multiple pathways and target genes. Future research should further investigate these pathways and target genes for a better understanding of the pharmacological mechanisms of WDD in treating SD.

In this study, a comprehensive network of compound-target interaction was established, and the compounds of WDD that act on SD were identified. Our results revealed the association of 70 active target genes with 34 active compounds, corresponding to different herbs in the WDD. Therefore, we hypothesized that every herb in the WDD has a function in responding to SD. Interestingly, active compounds in RP were found to perform a critical function in the compound-target network, consistent with the TCM theory of RP being the predominant active herb in WDD. The key compounds identified in the study were luteolin, naringenin, nobiletin, baicalein, beta-sitosterol, isosinensetin, tetramethoxyluteolin, stigmasterol, cavidine, and sinensetin. Luteolin has been shown to have clinical significance in the treatment of age-associated hearing loss caused by oxidative stress (Zhu *et al.*, 2021), while baicalein may protect auditory function in noise-induced hearing loss (NIHL) (Kang *et al.*, 2010). Thus, the impact of WDD on SD might be the consequence of the synergistic interactions among multiple compounds. However, further studies are needed to investigate the individual and combined effects of these

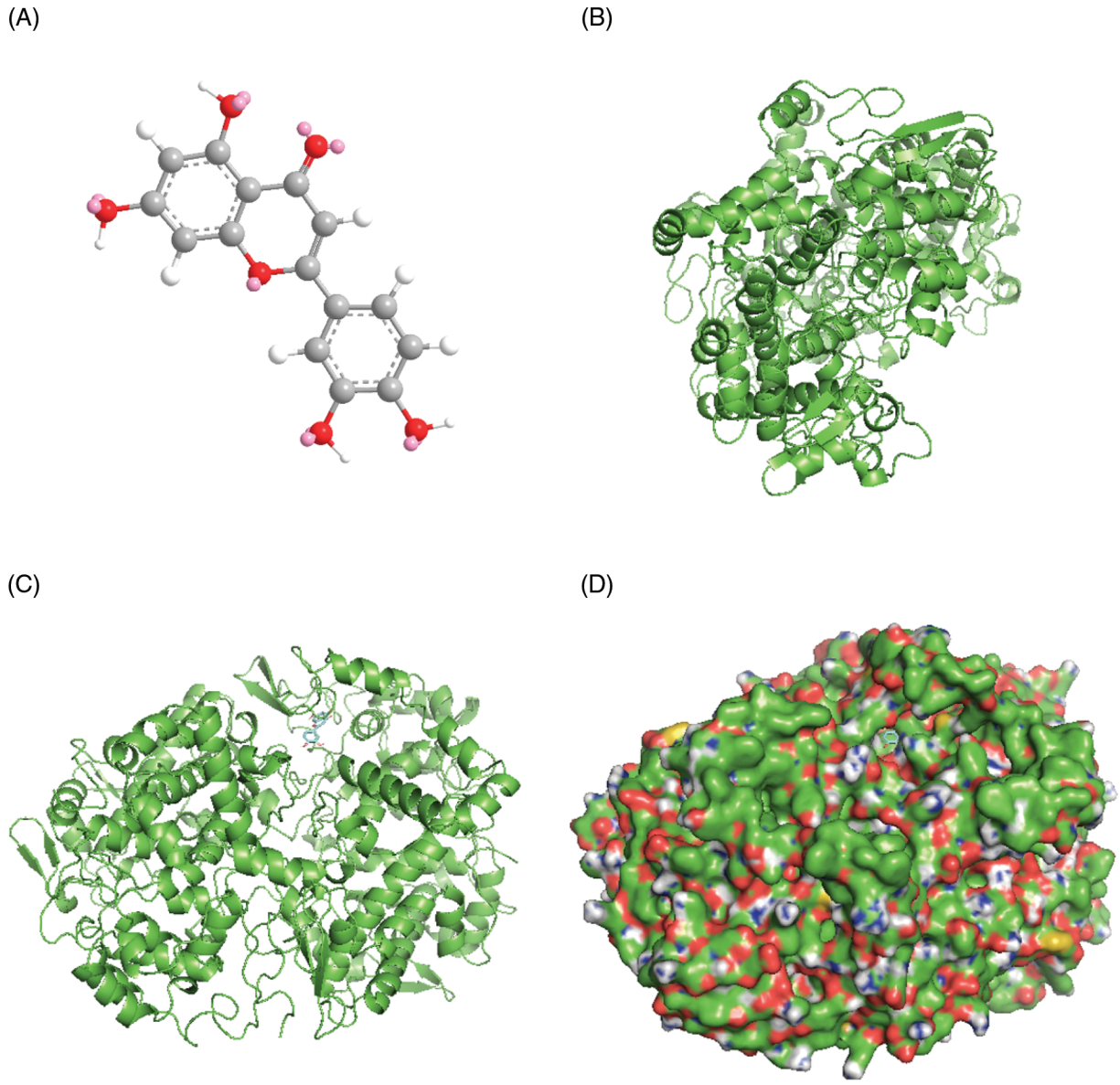


FIGURE 11. Molecular docking of caspase3 (CASP3) and baicalein. (A) Three-dimensional structures of baicalein from the PubChem database. (B) Three-dimensional structures of CASP3 from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) database. (C) Molecular docking simulation. (D) The surface of the protein is shown in a molecular docking simulation.

compounds on SD. Nonetheless, only a few studies have been conducted on the effects of these compounds on SD, which means that further research is needed in this field.

The PPI network highlighted that the impact of WDD on SD was linked to several different targets, including *AKT1*, *CASP3*, *TP53*, *JUN*, *PTGS2*, *VEGFA*, *MAPK3*, *ESR1*, *HIF1A*, and *EGFR*. Studies have shown that the activities of the phosphoinositide-3 kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase (MAPK) signaling pathways are crucial in the onset and survival of auditory hair cells in sudden sensorineural hearing loss (Liu *et al.*, 2021); Akt1, Akt2, and Akt3 are all implicated in the normal function of the ear, with Akt2 and Akt3 regulating the hair cell survival within the inner ear of mammals (Brand *et al.*, 2015). Polymorphisms in CASP3 have been linked to the risk of developing NIHL (Wu *et al.*, 2017) and mice lacking caspase-3 display hearing loss due to the loss of spiral ganglion and cochlear hair cells (Makishima *et al.*, 2011). In the zebrafish lateral line, p53, Bcl2, and Bax modulate neomycin- and gentamicin-mediated hair cell apoptosis (Coffin *et al.*, 2013). Hearing loss and auditory hair cell death caused by aminoglycosides and acoustic stress are prevented by a peptide antagonist of c-Jun N-terminal kinase (Wang *et al.*, 2003). PTGS2 performs a function in the etiology of NIHL, and pharmacological suppression of PTGS2 has been shown to have therapeutic significance in the treatment of this condition (Sun *et al.*, 2016). Vascular endothelial growth factor A signaling can generate new vessels in the ear (Wang *et al.*, 2019). *WBP2* codes for the WW domain-binding protein 2 (Wbp2), which functions as a transcriptional coactivator for estrogen receptor alpha (ESR1), and Wbp2 deficiency causes a

gradual loss of high-frequency hearing in mice (Buniello *et al.*, 2016). Hypoxia-inducible factor 1-alpha is involved in the protection against noise-induced inner-ear damage (Chung *et al.*, 2011). Our results reveal an association between these genes and SD, paving the way for additional investigation into the pharmacologic processes and underlying mechanisms.

We developed a key compound–target network and performed molecular docking to investigate specific interconnections between key compounds and their target proteins, which may potentially increase the reliability of the network model. The results of molecular docking studies revealed that the key active compounds in WDD exhibited strong binding abilities with their respective target proteins. By targeting similar signaling pathways, these active compounds might be able to enhance the therapeutic role of WDD in SD. Future research may explore compounds associated with the matched target proteins.

In this study, we utilized a network pharmacological method to explore the impact of WDD on SD for its pharmacological mechanisms and examined the interaction of the target with the relevant compound using molecular docking. Nonetheless, the research still has some notable constraints. First, the screening parameters and definitions employed to identify active compounds and gene targets associated with WDD in the TCMSP were fixed. The genes associated with SD were examined using five databases. Even though these databases are rather comprehensive, certain compounds and target genes might have been missed. Additionally, relevant data for CBT are currently lacking in the TCMSP database. But our main purpose was to find the primary compounds and targets for preliminary research, as our results showed that the main compounds and main targets, which could provide the basis for further mechanistic studies. Second, the fact that WDD is a mixture makes it challenging for other researchers to replicate experiments. However, HPLC studies were performed to facilitate the quantification of compounds in WDD, and HPLC studies on key compounds can be carried out to further validate the findings (Wang *et al.*, 2021; Zhang *et al.*, 2019). We can also start by studying the key compounds first. Third, further empirical analysis of the candidate target genes and pathways is necessary although GO functional enrichment and KEGG pathway enrichment analyses were conducted, and a PPI network was established to examine the gene targets and pathways of WDD that act on SD. Finally, although exploratory docking analyses were performed in this study, additional experimental investigations of small-molecular compounds and macromolecular target proteins are required to validate these findings in the future.

Conclusion

In this study, a network pharmacological approach was employed to investigate the multi-compounds, targets, and pathway mechanisms underlying the therapeutic effect of WDD on SD. Furthermore, molecular docking analysis of key compounds and their matching protein targets indicated that these compounds could serve as major modulators of the therapeutic action of WDD on SD. Overall, our study provides valuable insights into the underlying mechanisms of WDD in treating SD and may contribute to the development of novel therapeutic strategies for this condition.

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Author Contributions: Shiming Ye, Yufeng Zhang, Zheng Yan, Haibing Hua, and Wandong She conceptualized and designed the study and drafted and edited the manuscript. Shiming Ye, Yufeng Zhang, Ting Liu, Cong Wang, and Haibing Hua collected and extracted data from various sources and performed data analysis. Zheng Yan, Haibing Hua, and Wandong She were in charge of project administration and supervision. The final version was reviewed and approved by all authors.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article (and its supplementary files).

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare they have no conflicts of interest to report regarding the present study.

Supplementary Materials: The supplementary materials are available online at https://doi.org/10.32604/biocell.2023.029333.

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