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ARTICLE

The mechanism of Jiaweisinisan in the treatment of liver fibrosis: A study based on network regulation and molecular docking technology

Li-Qin Chao[1], Dong-Dong Yu[1], Jun-Xia Zhang[1*]

[1]Henan University of Traditional Chinese Medicine, Zhengzhou 450046, China.

***Corresponding to**: Jun-Xia Zhang. Henan University of Traditional Chinese Medicine, Zhengzhou 450046, China. E-mail: 363596126@ qq.com.

Abstract: Objective: To explore the potential targets and molecular mechanisms of action of Jiaweisinisan (JWSNS) in liver fibrosis intervention and treatment using network pharmacology and molecular docking technology, with the goal of providing a theoretical basis for future scientific research and clinical applications.
Methods: We used the TCMSP database to obtain the main active ingredients of the eight traditional Chinese medicines in JWSNS and screen the active ingredients with oral bioavailability ≥ 30% and druglikeness ≥ 0.18; moreover, we used PubChem database, Swiss Target Prediction, and SEA database to predict the potential targets of the active ingredients. DisGeNET database was used to search and screen for liver fibrosis disease targets. After mapping component and disease targets, Cytoscape 3.2.1 software was used to construct an active component-target network. We used STRING database to construct protein-protein interaction network and analyze potential protein functional modules. FunRich database was used to analyze biological processes, biological pathways, clinical phenotypes, cell structures, and molecular functions. Finally, PDB database, DockThor, and PyMol software were used for molecular docking.
Results: There are 192 active ingredients in JWSNS, 18,582 targets, and 255 targets for liver fibrosis mapping. Using STRING database and ClusterONE cluster analysis, 18 potential targets were selected, and 13 active ingredients were screened out by Cytoscape topology parameters. Potential targets were mainly involved in signal transduction, cellular immunity, cell cycle regulation, cell apoptosis, and other biological processes. They mainly acted on signal transduction events mediated by proteoglycan synthesis, neuroamine 1-phosphate (S1P) pathway, and PAR1 mediation. Signal pathways such as thrombin signaling events. The results of molecular docking showed that the main active ingredients had a greater affinity for PIK3CA.
Conclusion: JWSNS acts on immune response, apoptosis, signal transduction, and other reaction-related targets and pathways, and has a multi-component, multi-target, and multi-channel therapeutic effect on liver fibrosis. Its main active ingredients have the potential to fight liver fibrosis.
Key words: network regulation, molecular docking simulation, Jiaweisinisan, hepatic fibrosis, potential targets, signaling pathways

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***Abbreviations*:** HF, Hepatic fibrosis; JWSNS, Jiaweisinisan; PPI, protein-protein interaction; OB, oral bioavailability; DL, druglikeness; SMILES, simplified molecular-input line-entry system; S1P, sphingosine-1-phosphate; PI3K, phosphatidylinositol 3-kinase.

***Authors' Contributions*:** Conception and design: Junxia Zhang; Liqin Chao; Administrative support: Dongdong Yu; Provision of study materials or patients: Junxia Zhang; Collection and assembly of data:Liqin Chao; Data analysis and interpretation: Liqin Chao, Dongdong Yu. All authors read and approved the final manuscript.

***Competing interests*:** The authors declare that they have no conflict of interest.

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1. Introduction

Hepatic fibrosis (HF) is a pathological wound-healing response to chronic stimuli, and is characterized by an excessive deposition of collagen-based extracellular matrix in the liver, which results in a diffuse deposition of various collagen types in the liver, destruction of the reticular structure of the basement membrane, and capillarization of the hepatic sinusoids, eventually leading to progressive fibrotic liver lesion formation. HF has been currently considered as an inevitable yet reversible early stage of hepatic cirrhosis, and it can progress to irreversible hepatic cirrhosis if not treated in time. Globally, more than 1 million people die every year from end-stage liver disease caused by HF [1]. Presently, there are no specific drugs for the clinical treatment of HF, and drugs for single-targeted therapies have considerable side effects with low therapeutic efficacy [2]. Recently, our research team has been using Jiaweisinisan (JWSNS), a traditional Chinese herbal compound, to treat HF, and we found that JWSNS has an excellent therapeutic efficacy in both animal experiments and clinical applications without toxic side effects [3-11]. Given the wide range of clinical phenotypes of hepatic diseases treated by JWSNS [12], it is necessary to further explore the mechanism of action of JWSNS. Therefore, we performed big data mining based on network pharmacology and bioinformatics technology, which provided more insights into the anti-HF effect of JWSNS.

2. Methods

2.1 Materials

Data used in this study were retrieved from the following databases: TCMSP (http://tcmspw.com/tcmsp.php), a database of systems pharmacology for drug discovery from herbal medicines; DisGeNET (https://www.disgenet.org/), a database of disease genes and their mutation sites; UniProt (https://www.uniprot.org/), a database of protein targets; STRING (https://string-db.org/cgi/input.pl), a protein-protein interaction (PPI) database; PDB (http://www.rcsb.org/pdb/home/home.do), a database of RCSB protein; and PubChem (https://pubchem.ncbi.nlm.nih.gov/). Data analysis was performed using the following software: Cytoscape 3.2.1, FunRich (http://www.funrich.org/), molecular docking software DockThor (https://dockthor.lncc.br/v2/), and PyMol (for visualization of molecular docking results).

2.2 Screening and target prediction of JWSNS ingredients

TCMSP was searched for JWSNS pharmacological ingredients with oral bioavailability (OB) ≥ 30% and druglikeness (DL) ≥ 0.18 using the eight medicinal materials in JWSNS as keywords, namely *Bupleurum* (Chai Hu), *Fructus Aurantii Immaturus* (Zhi Shi), *Radix Paeoniae Alba* (Bai Shao), *Licorice* (Gan Cao), *Codonopsis pilosula* (Dang Shen), *Atractylodes* (Bai Shu), *Poria* (Fu Ling), and *Kelp* (Kun Bu), leading to four effective ingredients. The simplified molecular-input line-entry system (SMILES) notations of each active ingredient were searched in PubChem database. Human genes of the target proteins were retrieved using Swiss Target Prediction (a web server for the target prediction of bioactive small molecules), SEA database, and UniPort database.

2.3 Prediction and network construction of targets for the anti-HF effect of JWSNS

The target proteins of HF and the encoding genes were retrieved from DisGeNET using the keyword “hepatic fibrosis” The intersection of drug targets and disease targets was obtained based on the UNIPROT numbers and was taken as the potential targets of JWSNS for HF prevention and treatment. Cytoscape 3.2.1 was used to construct an active ingredient-target interaction network, whose topological parameters were analyzed using Network Analyzer.

2.4 PPI network construction

The common targets of JWSNS and HF were imported into STRING to screen for human (PPI) with high confidence (> 0.9) while removing isolated target proteins, and the results were used to construct a PPI network. The PPI data were imported into Cytoscape 3.2.1 for further analysis with the ClusterONE plug-in, leading to the identification of potential protein functional modules, which were analyzed using FunRich, a functional enrichment analysis software, to identify the biological processes and functions of the modules.

2.5 Target function and pathway enrichment analysis

In order to gain deeper insights into the biological processes and signaling pathways that may involve target protein-encoding genes, we imported targets for the anti-HF effect of JWSNS into FunRich to identify a significant enrichment ($P < 0.01$) of target genes in various biological processes, biological pathways, clinical phenotypes, cellular structures, and molecular functions in the human body. Clustering analysis results were visualized.

2.6 Molecular docking

We downloaded files containing the 3D structures of phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) in pdb format and small active molecules in sdf format from the RCSB PDB database (https://www.rcsb.org/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/), respectively. Online processing was performed through DockThor (https://

dockthor.lncc.br/v2/), and the results were imported into PyMOL software for visualization and analysis.

3. Results

3.1 Active ingredients and targets of JWSNS

A total of 981 active ingredients of JWSNS were retrieved through search in TCMSP, which were screened against the criteria of OB ≥ 30% and DL ≥ 0.18 based on the pharmacokinetic properties of the drug, leading to the obtainment of 8 medicinal materials of 192 active ingredients, among which 9 active ingredients were common to 2 or more medicinal materials. For the 192 active ingredients, a total of 18,582 targets were retrieved through search in TCMSP. The distribution of active ingredients and corresponding targets in the eight medicinal materials is presented in Table 1.

3.2 JWSNS targets for HF prevention and treatment

A total of 1,179 HF targets were retrieved through search in DisGeNET with "hepatic fibrosis" as the keyword. Intersection between the targets of active ingredients and the targets of the disease was acquired, revealing 971 targets that might be related to the anti-HF effect of JWSNS. Duplicate targets were removed from the 971 targets, leaving 255 mapped targets for subsequent analysis (Table 1).

3.3 Active ingredient-target network for the anti-HF effect of JWSNS

The circular nodes in Figure 1 represent the common targets, while the V-shaped nodes represent the active ingredients of JWSNS. Each node color is associated with a degree value, and a more reddish color indicates a higher degree value; yellow to red colors correspond to low to high degree values, respectively. A higher degree value (a more reddish color) indicates a larger number of nodes connected to a target in the network, and therefore a greater importance of the target. As shown in Figure 1, some single compounds in JWSNS act upon multiple targets, and some single targets are jointly acted upon by different compounds, which reflects the multi-ingredient and multi-target characteristics of traditional Chinese drugs. According to the topological parameters of the ingredient-target network (namely, the average shortest path length, degree, betweenness, and closeness of nodes), the main active ingredients responsible for the anti-HF effect of JWSNS were identified. The active ingredients not included in PubChem were removed, leaving eight active ingredients for subsequent analysis (Table 2), namely MOL004879, MOL004948, MOL004648, MOL004989, MOL000280, MOL008406, MOL001910, MOL004935, MOL004835, MOL004820, MOL004815, and MOL004814.

3.4 PPI Network of the targets for the anti-HF effect of JWSNS

The PPI network of the 255 common target proteins between the active ingredients and the disease was analyzed with STRING to screen for targets of high confidence (> 0.9) while removing isolated targets, leading to the identification of 174 targets and 921 interaction lines (Figure 2, Table 3). Module analysis was performed on the PPI network using the ClusterONE plug-in in Cytoscape 3.2.1, leading to the identification of one significant module with $P < 0.01$ (Figure 3, Table 4). Biological process enrichment analysis was performed on the genes of the module using FunRich. Five biological processes were identified by p-values (Figure 4).

Table 1. The number of active ingredients in JWSNS and the number of common targets between the active ingredients and HF

Medicinal material	Number of active ingredients	Number of active ingredients selected after screening	Number of targets	Number of common targets
Bupleurum (Chai Hu)	288	15	1500	132
Fructus Aurantii Immaturus (Zhi Shi)	65	22	414	70
Radix Paeoniae Alba (Bai Shao)	77	13	1173	121
Licorice (Gan Cao)	280	92	9165	218
Codonopsis pilosula (Dang Shen)	134	21	1992	163
Poria (Fu Ling)	34	15	1500	104
Atractylodes (Bai Shu)	55	7	436	70
Kelp (Kun Bu)	48	7	700	93
Total	981	192	18582	971

Note: 1. There are 1,179 targets of HF; 2. There are 255 mapped targets after removal of duplicate targets from the 971 targets of JWSNS

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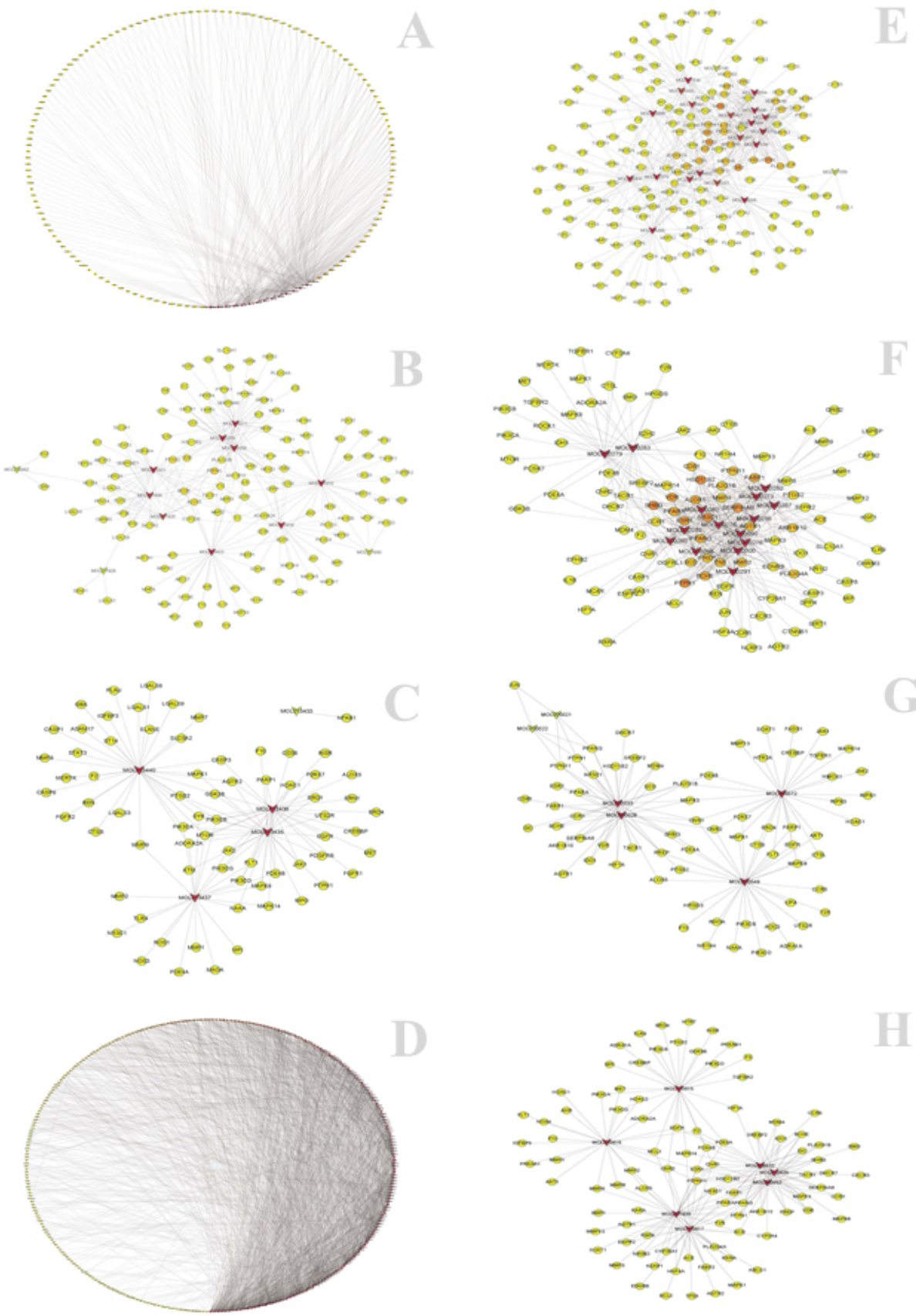

Figure 1. Active ingredient-target network for the anti-HF effect of the traditional Chinese drug JWSNS

Table 2. Topological parameters of main active ingredient nodes in the active ingredient-target network for the anti-HF effect of JWSNS

Ingredient name	Formula	Molecular weight	Average shortest path length	Betweenness	Closeness	Degree
MOL004879	$C_{22}H_{22}O_6$	382.4 g/mol	2.44805195	0.019125	0.40848806	40
MOL004948	$C_{21}H_{18}O_6$	366.4 g/mol	2.46103896	0.018576	0.40633245	38
MOL004648	$C_{33}H_{42}O_{19}$	742.7 g/mol	2.39726027	0.197245	0.41714286	37
MOL008406	$C_{39}H_{56}O_{12}$	716.9 g/mol	2.54098361	0.170052	0.39354839	36
MOL004935	$C_{20}H_{20}O_6$	356.4 g/mol	2.48051948	0.010511	0.40314136	35
MOL004835	$C_{20}H_{18}O_4$	322.4 g/mol	2.48051948	0.013487	0.40314136	35
MOL004815	$C_{17}H_{16}O_4$	284.31 g/mol	2.48051948	0.010524	0.40314136	35
MOL004814	$C_{16}H_{10}O_6$	298.25 g/mol	2.48701299	0.016831	0.40208877	35

Note: A *Bupleurum* (Chai Hu); B *Radix Paeoniae Alba* (Bai Shao); C *Fructus Aurantii Immaturus* (Zhi Shi); D *Licorice* (Gan Cao); E *Codonopsis pilosula* (Dang Shen); F *Poria* (Fu Ling); G *Atractylodes* (Bai Shu); H *Kelp* (Kun Bu)

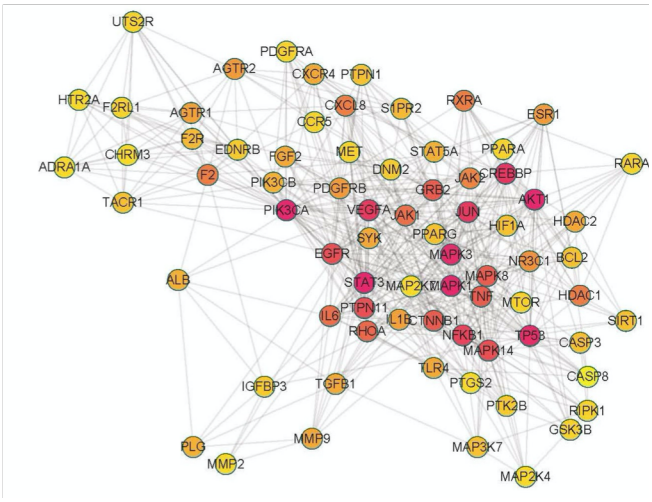

Figure 2. PPI network of the main targets for the anti-HF effect of JWSNS

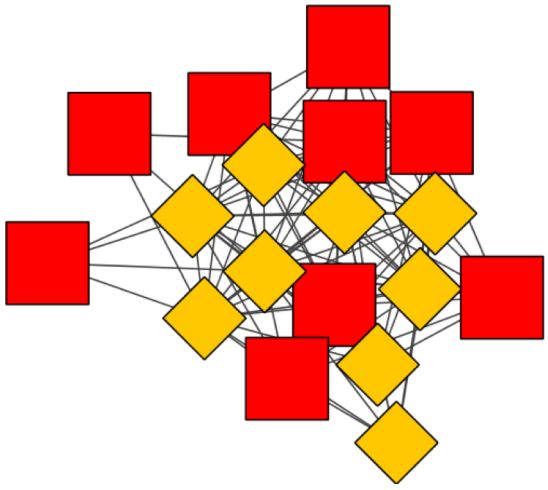

Figure 3. Clustering analysis diagram of the main targets for the anti-HF effect of JWSNS

Table 3. Topological parameters of main target nodes in a PPI network of targets that are involved in the anti-HF effect of JWSNS

Target	Average shortest path length	Betweenness	Closeness	Degree
PIK3CA	2.285	0.07821328	0.43763676	43
MAPK1	2.17	0.0764115	0.46082949	40
STAT3	2.205	0.058297	0.45351474	40
AKT1	2.185	0.07816149	0.4576659	38
MAPK3	2.195	0.04517689	0.45558087	37
TP53	2.24	0.08486305	0.44642857	36
CREBBP	2.225	0.07088192	0.4494382	34
JUN	2.27	0.05041019	0.44052863	33
VEGFA	2.255	0.05765975	0.44345898	33
NFKB1	2.265	0.07257369	0.4415011	31

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Table 4. Clustering parameters of the main targets for the anti-HF effect of JWSNS

Target	Average shortest path length	Betweenness	Closeness	Degree
PIK3CA	2.285	0.078213	0.4376368	43
STAT3	2.205	0.058297	0.4535147	40
PTPN11	2.435	0.027969	0.4106776	29
EGFR	2.375	0.03019	0.4210526	29
GRB2	2.43	0.012366	0.4115226	27
RHOA	2.425	0.016994	0.4123711	25
JAK1	2.48	0.008613	0.4032258	24
JAK2	2.515	0.019143	0.3976143	21
PDGFRB	2.63	0.004464	0.3802281	18
SYK	2.705	0.004457	0.3696858	16
FGF2	2.635	0.003611	0.3795066	16
STAT5A	2.625	0.005922	0.3809524	15
PIK3CB	2.745	0.001008	0.3642987	15
PTPN1	2.76	0.01085	0.3623188	13
PTK2B	2.69	0.002448	0.3717472	12
DNM2	2.75	0.006794	0.3636364	12
PDGFRA	2.85	0.00056706	0.3508772	11
MTOR	2.625	0.010411	0.3809524	11

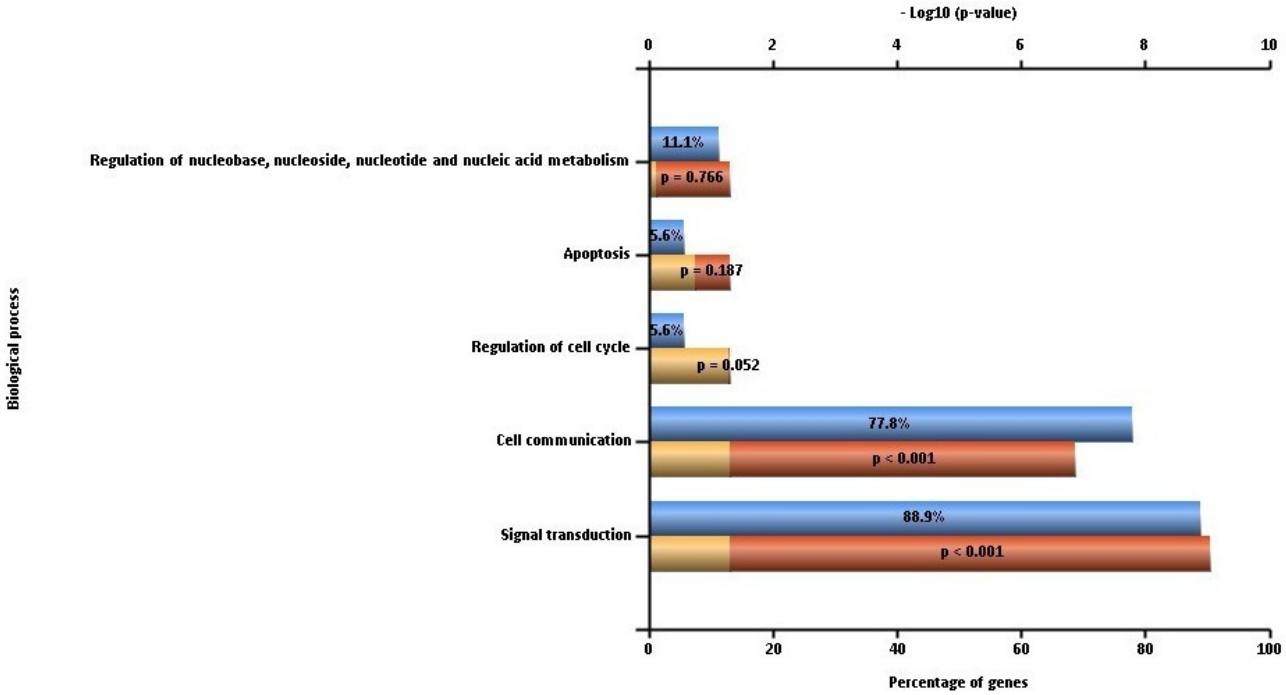

Figure 4. FunRich-based biological process enrichment analysis of the clustered target genes for the anti-HF effect of JWSNS

3.5 Enrichment analysis of the target functions and pathways involved in the anti-HF effect of JWSNS

Gene Ontology analysis was performed on the targets for the anti-HF effect of JWSNS using FunRich, leading to a retrieval of 126, 44, and 18 cellular composition, molecular function, and biological process terms, respectively. The enrichment analysis revealed: (1) JWSNS targets were mainly located in the cytoplasm, extracellular space, and plasma membrane (Figure 5A); (2) the functions of target molecules were mainly associated with the ligand-dependent nuclear receptor activity, transmembrane receptor protein tyrosine kinase activity, and metallopeptidase activity (Figure 5B); (3) and biological processes wherein the targets were involved mainly included signal transduction, cellular immunity, and cell apoptosis (Figure 5C). A total of 639 terms of biological signaling pathways were retrieved, mainly referring to signal transduction

events, sphingosine-1-phosphate (S1P) pathway, and PAR1-mediated thrombin signaling events (Figure 5D). A total of 1,572 clinical phenotype terms were retrieved, mainly referring to autosomal dominance, genetic heterogeneity, hyperplasia, and others (Figure 5E).

3.6 Molecular docking of the core ingredients for the anti-HF effect of JWSNS

The more stable the ligand-receptor complex conformation, the lower the binding energy. The top 2 core active ingredients in JWSNS were molecularly docked with the top 1 target of JWSNS. The molecular docking results showed that glycyrin bonded to PIK3CA by forming hydrogen bonds with two amino acids GLU-852 and ASP-793 in the vicinity of the active sites. Isoglycyrol bonded to PIK3CA by forming hydrogen bonds with two amino acids GLU-703 and GLN-782 in the vicinity of the active site. The molecular docking binding energies of glycyrin and isoglycyrol with PIK3CA were low, -7.41 kcal/mol and -8.187 kcal/mol, respectively, suggesting that the two active ingredients would efficiently bind to the PIK3CA target to form complexes with stable conformation (Figure 6).

4. Discussion

The core targets for the anti-HF effect of JWSNS were predicted to be associated with PIK3CA, MAPK1, STAT3, AKT1, MAPK3, TP53, CREBBP, JUN, VEGFA, and NFKB1. FunRich enrichment analysis revealed that the JWSNS targets were mainly distributed in the cytoplasm, extracellular space, and plasma membrane. The functions of the target molecules were mainly associated with the ligand-dependent nuclear receptor activity, transmembrane receptor protein tyrosine kinase activity, and metallopeptidase activity, and were mainly involved in the biological processes of signal transduction, cellular immunity, and cell apoptosis. There were 639 terms of biological pathways, mainly referring to signaling pathways, which included signal transduction events, S1P pathway, and PAR1-mediated thrombin signaling events. There were 1,572 clinical phenotype terms, mainly referring to autosomal dominance, genetic heterogeneity, hyperplasia, and others.

Network clustering analysis through the ClusterONE plug-in revealed that the core targets of JWSNS were PIK3CA, STAT3, PTPN11, EGFR, GRB2, RHOA, JAK1, JAK2, PDGFRB, SYK, FGF2, STAT5A, PIK3CB, PTPN1, PTK2B, DNM2, PDGFRA, and MTOR, all of which were involved in signal transduction, cellular immunity, cell cycle regulation, cell apoptosis, and other life processes. Computation revealed that the top two core active ingredients of JWSNS were glycyrin and isoglycyrol, and the top core target was PIK3CA.

PIK3CA gene encodes the catalytic subunit p110 alpha of class I phosphatidylinositol 3-kinase (PI3K). The family of PI3Ks is involved in the regulation of

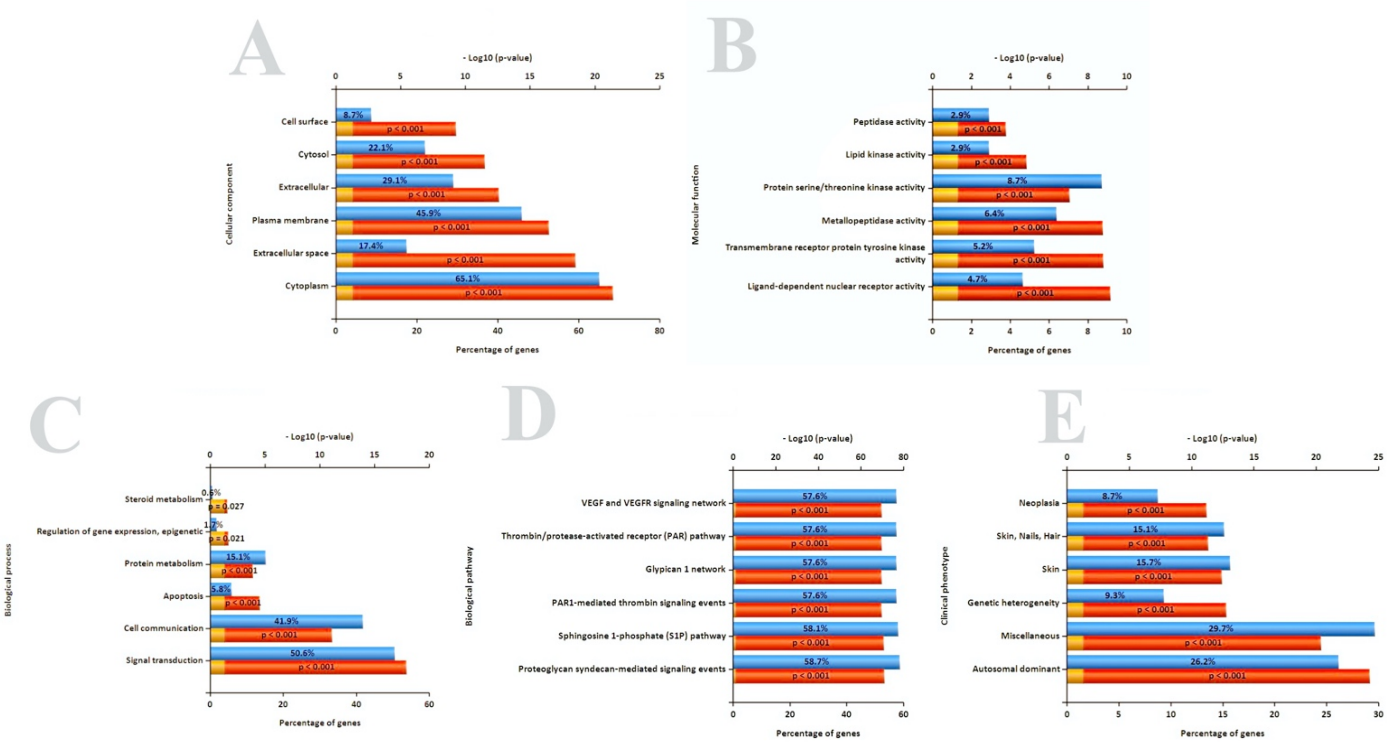

Figure 5. FunRich enrichment analysis of the target genes for the anti-HF effect of JWSNS. A. cellular component; B. molecular function; C. biological process; D. signaling pathway; E. clinical phenotype.

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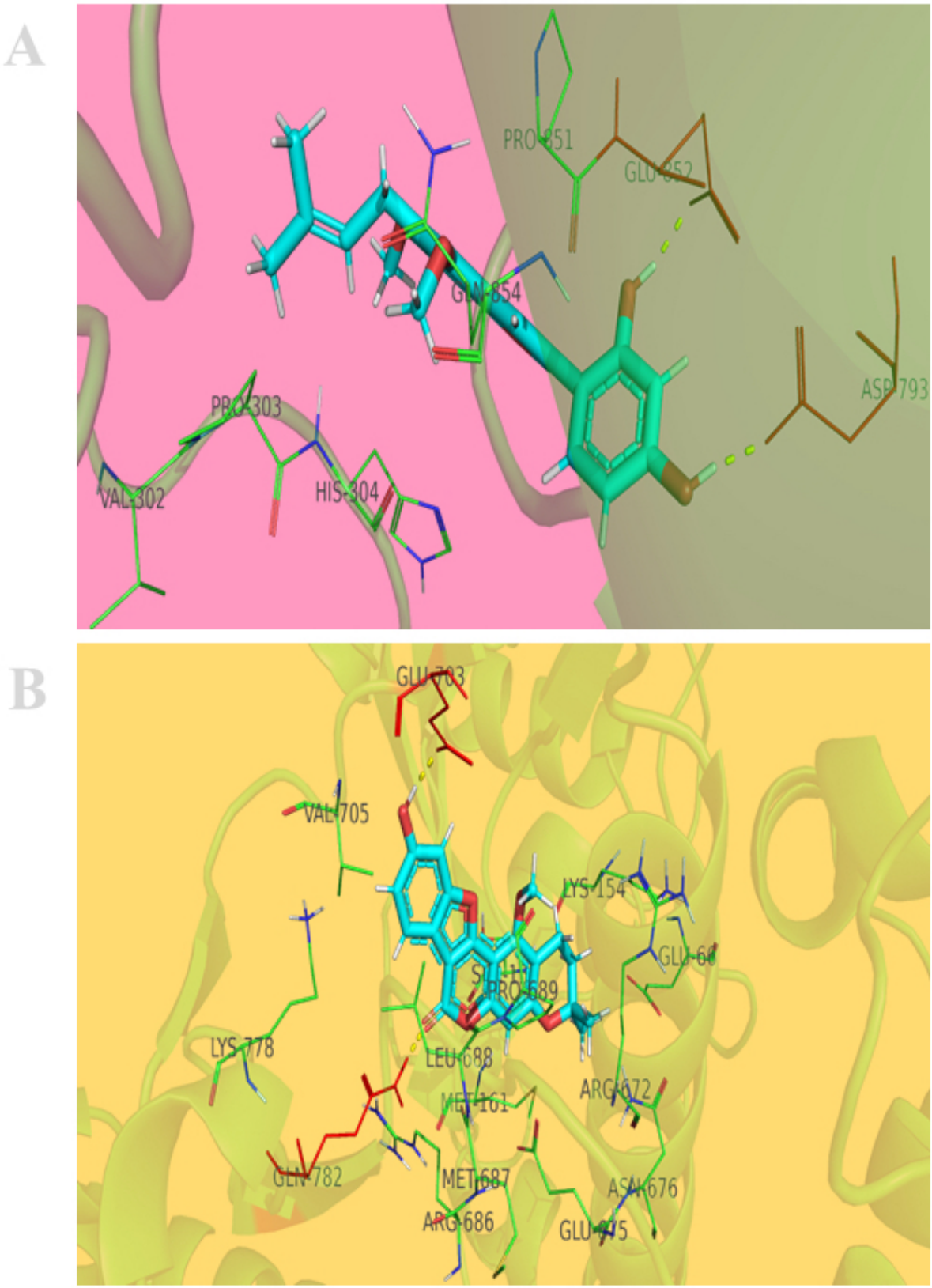

Figure 6. Molecular docking of the two core ingredients (glycyrin and isoglycyrol) of JWSNS with the PIK3CA target. A. Molecular docking of glycyrin with the PIK3CA target; B. Molecular docking of isoglycyrol with the PIK3CA target.

multiple cellular functions, including cell proliferation, apoptosis, differentiation, invasion, and metastasis. An abnormally up-regulated expression of PIK3CA will enhance the catalytic activity of PI3K, which in turn activates the PI3K/Akt signaling pathway, leading to excessive cell proliferation, adhesion, and differentiation while affecting cytoskeleton rearrangement and intracellular transport [13-15].

Molecular docking was conducted to further verify the mechanism of the anti-HF effect of JWSNS. The results showed that the core active ingredients (glycyrin and isoglycyrol) of JWSNS could well bind to the PIK3CA target, further confirming the anti-HF potential of JWSNS.

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