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Research Article

Natural Bioactive Compounds Promote Cell Apoptosis in Gastric Cancer Treatment: Evidence from Network Pharmacological Study and Experimental Analysis

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Background. Gastric cancer (GC) is one of the most lethal cancers. Shenlian capsule (SLC) is a Chinese patent medicine made from 11 herbs containing numerous plant-derived compounds, and the clinical trials of SLCs confirmed that they had effective adjuvant therapy for a variety of cancer such as lung cancer and gastric cancer. Moreover, the HPLC fingerprint of SLCs was established from other research to find potential components. In this study, network pharmacology-based research was used to identify combinations with molecules, targets, and pathways to explore their interaction mechanisms. *Methods*. The Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and the Traditional Chinese Medicine Integrated Database (TCMID) were widely implemented in selecting the active chemical components of SLCs with an oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 18%. In addition, the TCMSP and TCMID databases obtained the targets of SLCs, and PharmMapper (PM) was used to predict targets of SLCs. Gastric cancer-related genes were provided by the GeneCards and TTD databases. Subsequently, the drug/target/pathway network was established and visualized using Cytoscape software. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) enrichment analyses were used to predict the potential genes and pathways of gastric cancer. Molecular docking was performed to study the interaction between ligands and targets; the interaction was visualized using Discovery Studio and PyMOL. Finally, the potential primary mechanism used by SLCs against gastric cancer was verified by cell experiments, including MTT cell apoptosis assay, flow cytometry cell cycle assay, and western blotting with HGC-27 cells (undifferentiated). *Results*. Of 213 active chemical components from SLCs, 35 primary active chemical components were identified, and 10 potential critical targets were selected from the 185 intersections of the targets of SLCs and GC, such as RAC-alpha serine/threonine kinase 1 (AKT1), cellular tumor antigen p53 (TP53), interleukin-6 (IL6), caspase-3 (CASP3), vascular endothelial growth factor A (VEGFA), and epidermal growth factor receptor (EGFR). GO and KEGG enrichment analysis provided the PI3K/AKT, TNF, and p53 signaling pathways, which may be the primary signaling pathways modulating gastric cancer. Molecular docking verified targets such as AKT1, TP53, EGFR, and CASP3, which exhibited satisfactory binding capacity with active ingredients. Experiments with HCG-27 cells confirmed that SLCs may provide favorable treatment for GC by promoting CASP3 and TP53 expression to induce cell apoptosis and provided the predictions for network pharmacology and molecular docking. MTT and flow cytometry assays verified that SLCs promoted cell apoptosis and inhibited cell proliferation by triggering G0/G1 and S cell cycle arrest. In addition, western blot analysis confirmed that SLCs promoted TP53 and CASP3 overexpression, which led to HGC-27 gastric cell apoptosis. *Conclusions*. Our results confirmed that SLCs inhibit proliferation of HGC-27 gastric cell by promoting cell apoptosis and, therefore, have potential in the treatment of advanced gastric cancer. P53 signaling pathway was the key pathway. In addition, quercetin, matrine, and ursolic acid might be the main active ingredients.

1. Introduction

Gastric cancer (GC), a common malignant tumor, has become the fourth most lethal cancer worldwide, as reported in the 2020 GLOBOCAN project by the International Agency for Research on Cancer (IARC) [1]. Gastric cancer is classically divided into intestinal and diffuse histologic types, and most cases of gastric cancer were induced by infection with the bacterium *Helicobacter pylori* [2]. Currently, patients with advanced gastric cancer usually choose

chemotherapy and radiotherapy as the major treatment due to a lack of effective surgical treatments and a late diagnosis. Despite this, the survival rate for patients with advanced gastric cancer remains dismal [3, 4]. Doxorubicin hydrochloride (Dox), irinotecan (CPT-11), and esophagogastroduodenoscopy (EGD) are commonly used to treat gastric cancer. However, drug resistance is frequently observed in the treatment of GC, and therefore, it is necessary to find new and effective drugs for treatment. Natural bioactive compounds have potential as a cancer therapy; therefore, the uses of natural bioactive compounds have become one of the hottest topics worldwide.

Traditional Chinese medicine (TCM) has been widely used in the treatment of cancer because natural bioactive compounds have the capability of exerting synergistic effects upon multiple targets [5–7]. To exemplify, Xiao-Ai-Ping (XAP) injection has been effectively used to treat gastric cancer and lung cancer [8, 9], and Weikang Keli has also been used for gastric cancer therapy in cell experiments [10]. Thus, there is promising potential for TCM to be used to treat advanced gastric cancer.

Shenlian capsule (SLC) is a Chinese patent medicine that is prepared from 11 Chinese herbs: Danshen (Radix Salviae Miltiorrhizae/Chinese salvia (Salvia miltiorrhiza Bunge)), Kushen (Radix Sophorae Flavescentis Radix/shrubby sophora (*Sophora flavescens* Aiton)), Banzhilian (Herba Scutellariae Barbatae/barbed skullcap (Scutellaria barbata D. Don.)), Shandougen (Radix et Rhizome Sophorae Tonkinensis/bushy sophora (Sophora tonkinensis Gagnep.)), Baibiandou (Semen Lablab Album/hyacinth bean (Dolichos lablab L.)), Sanleng (Rhizoma Sparganii/common bur-reed (Sparganium stoloniferum Buch.-Ham.)), Ezhu (Rhizoma Curcumae (Zedoariae)/zedoary root (Cucurma zedoaria (Christm.) Roscoe), Fangji (Radix Stephaniae Tetrandrae/stephania root (Stephania tetrandra S. Moore)), Wumei (Fructus Mume/dried black plum (Prunus mume (Sieb. et Zucc.))), Buguzhi (Fructus Psoraliae/babchi (Psoralea corylifolia L.)), and Kuxingren (Semen Armeniacae Amarum/apricot seeds (Prunus armeniaca L.)). SLCs have already been used for the treatment of lung cancer and gastric cancer. The high performance liquid chromatography (HPLC) fingerprint of Shenlian also established by Han' s team to find the potential ingredients of SLCs. Nine components were identified such as oxymatrine, matrine, tetrandrine, fangchinoline, amygdalin, oleanolic acid, ursolic acid, psoralen, and angelicin [11–13]. Several clinical trials in China have confirmed that adjuvant treatment with SLCs was effective with bevacizumab injection, epirubicin hydrochloride injection, cisplatin injection, or fluorouracil injection for advanced gastric cancer [14, 15]. However, the treatment and action mechanisms of the SLC remain undefined. Thus, a network pharmacology-based study, which was proposed by Hopkins and relies on network database retrieval, system biology, and pharmacology, should be undertaken to elucidate SLCs' potential treatment mechanism in gastric cancer [16–18]. Network pharmacology-based studies have the ability to determine the primary potential ingredients, targets, and pathways involved [19, 20].

To further identify the mechanism of action of the SLCs, molecular docking was used to validate combination ingredients with targets [21]. The binding energy and bond interacitons between ingredients and targets interpreted the biological activity of the SLCs' main ingredients.. By also conducting MTT cell apoptosis assay, flow cytometry, and western blot analysis, the specific mechanisms used by the SLC to regulate gastric cancer cell apoptosis, cell cycle arrest, and their signal transduction pathways were elucidated [22]. Finally, the mechanisms used by the SLC against GC were clarified through the above research, and a schematic flow diagram is shown in Figure 1.

2. Methods

2.1. Main Active Ingredients and Target Identification. The main active chemical ingredients in all the herbs in the SLC were found in the Traditional Chinese Medicine Systems Pharmacology (TCMSP; https://tcmsp-e.com/tcmsp.php) database and Traditional Chinese Medicine Integrated Database (TCMID; https://www.megabionet.org/tcmid/). These components were identified based on oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 18%. The targets of the main components were obtained from TCMSP and TCMID, and the predictions of the SLC's potential targets were obtained from the PharmMapper database (PM; https://www.lilab-ecust.cn/pharmmapper/) based on a z score ≥4. The UniProt database (https://www.uniprot.org/) and DrugBank database (https://www.drugbank.ca) provided the human genes related to these targets. GC targets were selected from the GeneCards database (https://www.genecards.org/) and TTD database (https://bidd.nus.edu.sg/group/ttd/ttd.asp) by searching using the keywords "gastric cancer," "stomach cancer," and "gastric carcinoma."

2.2. Drugs/Targets/Pathways and Protein-Protein Interaction Network Construction. The targets and potential active ingredients were imported into Cytoscape (v. 3.8.0) for the construction of a drug/target/pathway network. By selecting high-degree targets and active ingredients, key targets and main active ingredients were mined. Protein-protein interaction (PPI) networks were constructed using STRING tools (https://string-db.org) and Cytoscape to find the potential leader targets.

2.3. GO and KEGG Enrichment Analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) enrichment analyses were applied from the DAVID database (https://david.ncifcrf.gov/) based on a P value < 0.05 as the condition to visualize the results and confirm key signaling pathways.

2.4. Molecular Docking Analyses. The 3D structures of proteins were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank database (https://www.rcsb.org/). The chosen scientific name of the source organism was *Homo sapiens*, and it was refined at resolutions of 1.0–3.0 A°. Ligands were downloaded from TCMSP and TCMID and generated using

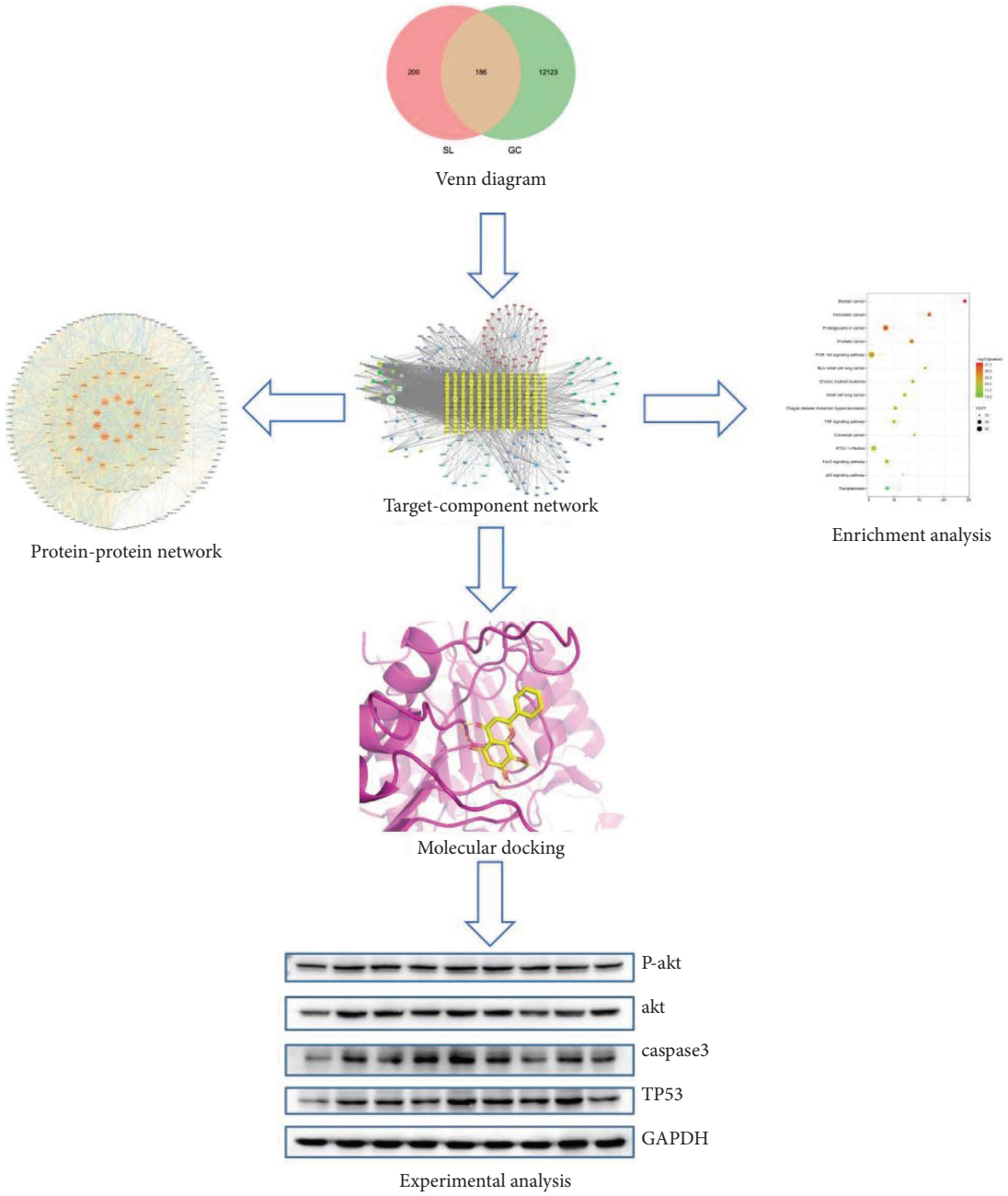


Figure 1: Schematic flow diagram showing the steps used for determining the active ingredients, network pharmacology analysis, molecular docking, and experimental analysis of SLCs.

ChemDraw (v. 14.0). AutoDockTools (v. 1.5.6) was used to prepare the input files, and AutoDock Vina was used to calculate the affinities between these proteins and ligands. Then, the docking results, which are docking binding energies based on the threshold conditions of ≤ -8.0 kcal/mol, were collected and were then integrated and visualized using PyMOL (v. 2.3.0) and Discover Studio (v. 2017).

2.5. Experimental Analysis

2.5.1. Cell Culture. HGC-27 (undifferentiated) gastric cells were provided by Procell Life Science & Technology (Wuhan, China). The cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium with fetal bovine serum (20%, FBS) (HyClone, China) and a mixture of 1% antibiotics (streptomycin 100 U/mL and penicillin 100 U/mL) in an incubator containing 5% CO_2 at 37°C. The culture medium was replaced every 2-3 days at both the apical and basolateral ends. After 90% confluence, the cells were subcultured on plates.

2.5.2. MTT Analysis. HGC-27 cells were digested with trypsin to produce a single-cell suspension, followed by their seeding in a 96-well plate at 3×10^4 cells/well. After incubation for 24 h, the cells were incubated with different

concentrations of SLC solution (0 g/L, 0.25 g/L, 0.5 g/L, 1 g/L, 2 g/L, and 4 g/L) in a constant temperature incubator at 37°C for 48 h. After the end of the culture, 100 μL MTT was added to each well. After 4 h, the MTT solution was removed, and 150 μL DMSO was added. The plate was then shaken for 10 min at a low speed, and the OD value was determined at 490 nm by an enzyme labeling instrument.

2.5.3. Investigation of Apoptosis and Cell Cycle through Flow Cytometry. The HGC-27 cells were divided into three groups: blank, control (SLC 0 g/L), and experimental (SLC 2 g/L and 4 g/L). Then, the HGC-27 cells were inoculated into a 6-well plate. After 48 h of corresponding treatment in an incubator containing 5% CO_2 at 37°C, cells from each group were collected and washed using phosphate-buffered saline (PBS), 0.5 mL trypsin was added, and the cells were then centrifuged. Flow cytometry was subsequently performed to analyze the cell cycle.

2.5.4. Western Blotting. The samples were lysed on ice for 20 min and centrifuged for 20 min (12,000 rpm, 4°C), and the supernatant was then removed. Then, the concentrations of proteins were measured using a BCA Protein Assay Kit (Solarbio, Beijing, China). After adjusting the protein concentration using 5× loading buffer and PBS, the samples were boiled at 95°C for 5 min and prepared for electrophoresis. After electrophoresis, electroblotting was performed to transfer the proteins to a polyvinylidene fluoride (PVDF) membrane. Five percent nonfat milk was used to block the PVDF membranes for 2 h, and then western blotting was performed with the primary antibodies against CASP3, Akt, phosphorylated Akt, and p53 at 4°C for 12 h. All of the primary antibodies were diluted in a ratio of 1 : 1000. After that, Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) was used to wash the PVDF membranes 3 times, for 10 min each time. Horseradish peroxidase (HRP)-conjugated secondary antibodies were diluted in a ratio of 1 : 1500 and incubated with the PVDF membranes for 1 h at room temperature. The PVDF membranes were then washed with TBST 3 times and 10 min each time. The enhancement solution in the ECL reagent was mixed with the stable peroxidase solution in a 1 : 1 ratio, and the immunoreactive protein bands were imaged by a fully automated chemiluminescence image analysis system. Then, TBST was used to wash the PVDF membranes 3 times, for 5 min each time, and the PVDF membranes were then recovered and regenerated. The PVDF membranes were blocked again and incubated with secondary antibodies, with the other parameters remaining unchanged. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for the gray value analysis.

3. Results

3.1. Main Active Ingredients and Target Identification. A total of 386 potential targets were identified from the TTD database and GeneCards database. After comparison with the targets of the SLC, 185 potential targets associated with GC and the SLC were obtained from the database screening and prediction, as shown in Figure 2.

There were 855 active components retrieved from the TCMSP database and the TCMID in which OB ≥ 30% and DL ≥ 18%. Some of the basic information about these active components is shown in Table 1.

3.2. Drug/Target/Pathway and Protein-Protein Interaction Network Construction. The active components and targets were input into Cytoscape to construct the network of "drugs/targets/pathways," as shown in Figure 3. Thirty-five main potential active ingredients were mined from the network, as shown in Table 2. It has already been shown that common ingredients such as quercetin, ursolic acid, and kaempferol possess anticancer activity. Thus, they might be the main potential ingredients. In addition, wogonin and moslosooflavone may also play a very important role in the treatment of GC.

The protein-protein interaction network diagrams were obtained from STRING tools and Cytoscape, as shown in Figure 4. The protein-protein interaction network diagrams indicated that AKT1, TP53, VEGFA, and CASP3 might be key targets.

3.3. GO and KEGG Enrichment Analysis. The main potential genes and pathways were determined using GO and KEGG enrichment analysis, as shown in Figures 5 and 6. The primary signaling pathways may be those of PI3K-Akt, TNF, FoxO, and p53. The pathways in cancer were the most significantly enriched. The PI3K-Akt signaling pathway regulates cell survival and cell cycle, the TNF signaling pathway, and the FoxO signaling pathway. The p53 signaling pathway regulates cell apoptosis. These pathways had well correlation with AKT1, TP53, CASP3, and EGFR that were the main potential genes of SLC because these pathways are highly involved in cancer development. The main potential ingredients of SLC like quercetin, matrine, ursolic acid, and stigmasterol can exert antitumor effects by altering cell cycle progression, inhibiting cell proliferation, promoting apoptosis, inhibiting angiogenesis and metastasis progression, and affecting autophagy found from previous studies. Hence, the main potential ingredients of SLC could regulate the expression of BP, CC, and MF to regulate cell apoptosis and cell cycle. The SLC regulated well with the expression of these pathways when administered for treatment for advanced gastric cancer.

3.4. Molecular Docking Analyses. Ten proteins and 35 ingredients were selected for the molecular docking analysis. Several results of molecular docking are shown in Table 3. The key targets such as AKT1, TP53, and CASP3 were able to strongly bind with quercetin, wogonin, and (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one, as shown in Figure 7. To exemplify, the binding energy between wogonin and TP53 was approximately −7.9 kcal/mol. Val147 and Pro223 were involved in pi-sigma bonding, and Cys220

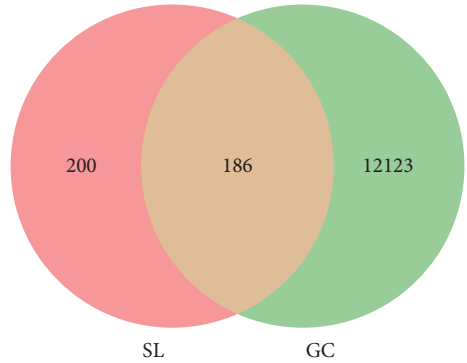


Figure 2: Venn diagram of common targets between the SLC and GC. After searching databases, 186 common targets were found.

Table 1: Potential ingredients in SLC (OB ≥ 30%, DL ≥ 0.18).

MOLID	Ingredients	OB (%)	DL
MOL008601	Methyl arachidonate	46.9	0.23
MOL000098	Quercetin	46.43	0.28
MOL000449	Stigmasterol	43.83	0.76
MOL000422	Kaempferol	41.88	0.24
MOL000511	Ursolic acid	16.77	0.75
MOL000263	Oleanolic acid	29.02	0.76
MOL001484	Inermine	75.18	0.54
MOL003627	Sophocarpine	64.26	0.25
MOL003648	Inermin	65.83	0.54
MOL003673	Wighteone	42.8	0.36
MOL003676	Sophoramine	42.16	0.25
MOL003680	Sophoridine	60.07	0.25
MOL000392	Formononetin	69.67	0.21
MOL004580	cis-Dihydroquercetin	66.44	0.27
MOL005944	Matrine	63.77	0.25
MOL006562	Leontalbinine	62.08	0.25
MOL006564	(+)-Allomatrine	58.87	0.25
MOL006566	(+)-Lehmannine	58.34	0.25
MOL006568	Isosophocarpine	61.57	0.25
MOL006571	Anagyrine	62.01	0.24
MOL006573	13,14-Dehydrosophoridine	65.34	0.25
MOL006582	5α,9α-Dihydroxymatrine	40.93	0.32
MOL006583	7,11-Dehydromatrine	44.43	0.25
MOL006596	Glyceollin	97.27	0.76
MOL003347	Hyperforin	44.03	0.60
MOL000006	Luteolin	36.16	0.25
MOL006613	Kushenin	47.62	0.38
MOL006619	Kushenol J	51.39	0.74
MOL006620	Kushenol J_qt	50.86	0.24
MOL006622	Kushenol O	42.41	0.76
MOL006623	Kushenol T	51.28	0.64
MOL006626	Leachianone G	60.97	0.40
MOL006627	Lehmanine	62.23	0.25
MOL006628	(+)-Lupanine	52.71	0.24
MOL006630	Norartocarpetin	54.93	0.24
MOL000456	Phaseolin	78.2	0.73
MOL006649	Sophranol	55.42	0.28
MOL006650	(−)-Maackiain-3-O-glucosyl-6′-O-malonate	48.69	0.52
MOL006652	Trifolirhizin	48.5	0.74
MOL001484	Inermine	75.18	0.54
MOL000354	Isorhamnetin	49.6	0.31
MOL003627	Sophocarpine	64.26	0.25
MOL003629	Daidzein-4,7-diglucoside	47.27	0.67
MOL003633	Oxynarcotine	56.74	0.60

Table 1: Continued.

MOLID	Ingredients	OB (%)	DL
MOL003647	Sophojaponicin	41.51	0.79
MOL003656	Lupiwighteone	51.64	0.37
MOL003663	Sophoranol	67.32	0.28
MOL003675	3,4,5,6-Tetradehydrospartein-2-one	71.26	0.24
MOL003677	Sophoranol	62.77	0.28
MOL000392	Formononetin	69.67	0.21
MOL007085	Salvilenone	30.38	0.38
MOL000915	(1S,10S),(4S,5S)-Germacrone-1(10),4-diepoxide	30.48	0.18
MOL000173	Wogonin	30.68	0.23
MOL001297	trans-Gondoic acid	30.7	0.20
MOL005030	Gondoic acid	30.7	0.20
MOL001735	Dinatin	30.97	0.27
MOL010922	Diisooctyl succinate	31.62	0.23
MOL007145	Salviolone	31.72	0.24
MOL006563	(+)-9-alpha-Hydroxymatrine	32.04	0.29
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41
MOL002331	N-Methylflindersine	32.36	0.18
MOL007143	Salvilenone I	32.43	0.23
MOL003644	Withaferine	33.14	0.73
MOL002719	6-Hydroxynaringenin	33.23	0.24
MOL002714	Baicalein	33.52	0.21
MOL007049	4-Methylenemiltirone	34.35	0.23

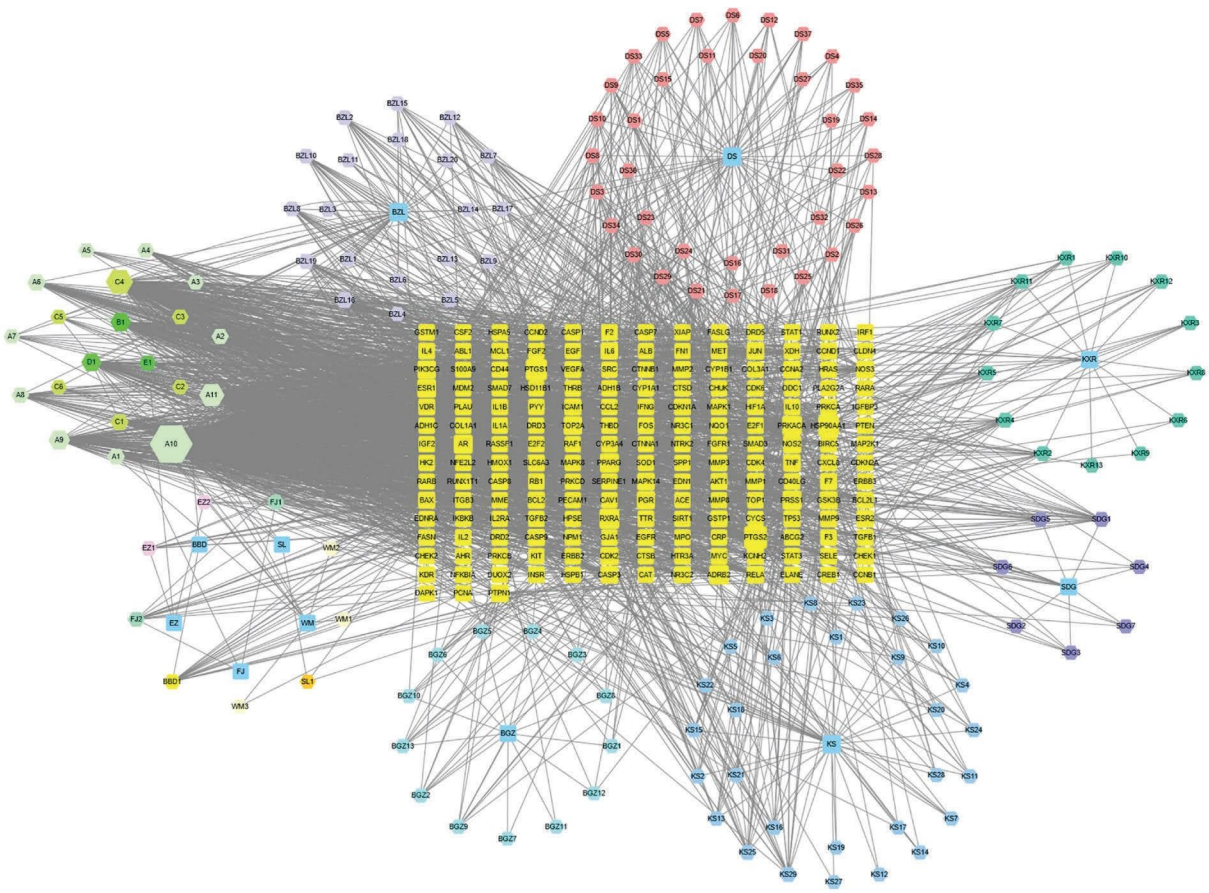
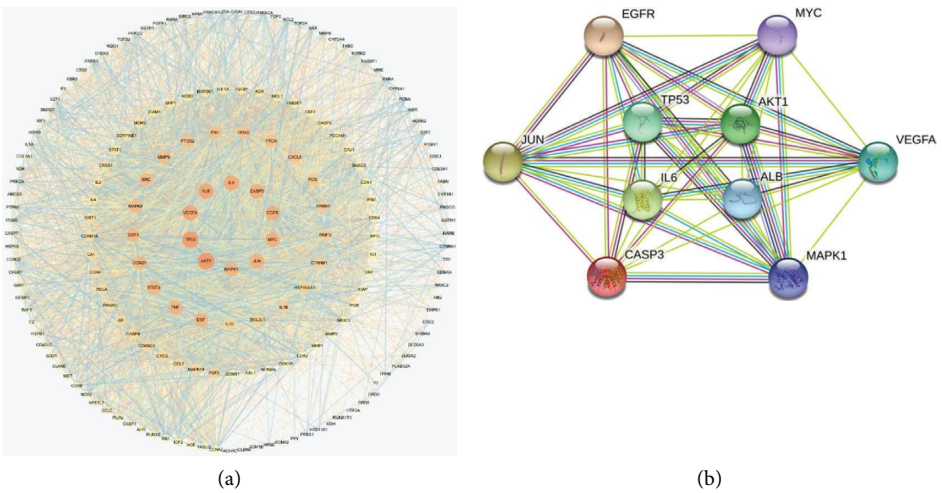


Figure 3: Drug/target/pathway network for SLC and GC. A1–A11, B1, C1–C6, D1, and E1 were common potential active ingredients. Other 21 potential ingredients like wogonin and rhamnazin had high degrees and betweenness that exceed the average value. Thus, 35 main potential active ingredients were mined for further research.

Table 2: Thirty-five main potential active ingredients in SLC.

Name	ID	Degree	Betweenness
Quercetin	A10	432	0.323217
Ursolic acid	C4	181	0.097642
Luteolin	A11	150	0.088354
Formononetin	A9	95	0.020403
Kaempferol	B1	74	0.038225
beta-Sitosterol	D1	68	0.015347
Wighteone	A6	42	0.005507
Wogonin	BZL16	36	0.039156
Stigmasterol	C1	33	0.012039
Matrine	A8	30	0.014486
(2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	A1	27	0.008905
Baicalein	BZL19	23	0.017185
Isorhamnetin	SDG1	22	0.011682
Tanshinone IIA	DS21	22	0.028999
Inermine	A2	20	0.003451
Inermin	A4	20	0.003451
beta-Carotene	BBD1	19	0.01471
8-Isopentenyl-kaempferol	KS29	18	0.006275
Rhamnazin	BZL8	18	0.0044
Moslosooflavone	BZL10	17	0.004829
Salvilenone	DS29	17	0.003735
Danshexinkun D	DS30	17	0.008629
Licochalcone B	KXR2	16	0.003991
Glabridin	KXR4	16	0.004015
Phaseolin	KS25	15	0.003611
5-Hydroxy-7,8-dimethoxy-2-(4-methoxyphenyl) chromone	BZL2	15	0.003367
Tetrandrine	FJ2	15	0.0194
Lupiwighteone	SDG5	15	0.002831
Rivularin	BZL15	14	0.004003
Hederagenin	E1	14	0.01254
Chrysin-5-methylether	BZL12	13	0.002947
Cryptotanshinone	DS3	13	0.008271
Tanshinone VI	DS24	13	0.004279
Miltirone	DS34	13	0.002486
Phaseol	KXR5	13	0.003233



(a) (b)

Figure 4: Protein-protein interaction of expression genes: (a) the combination of all types of expression genes and (b) 10 high-degree gene combinations.

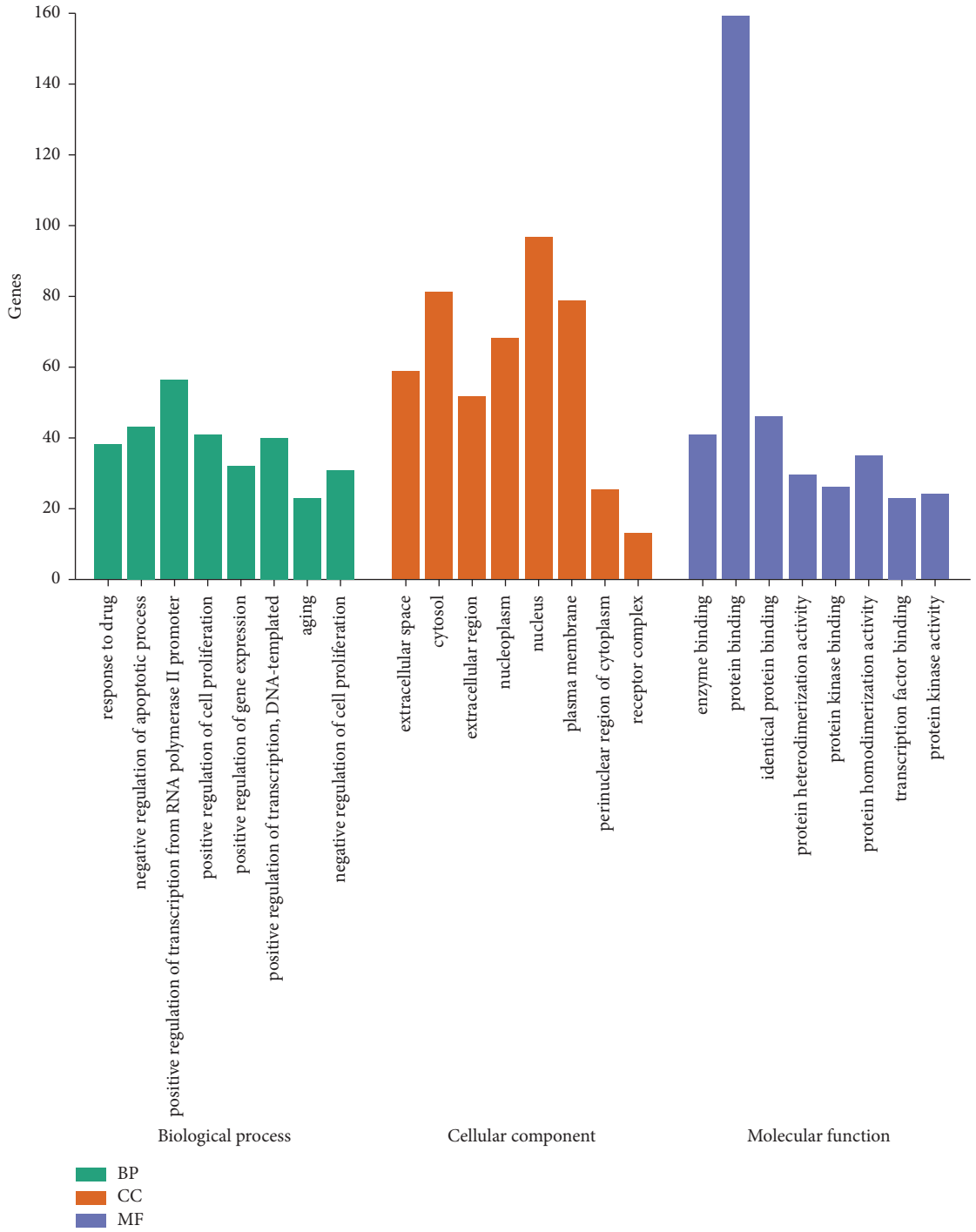


Figure 5: GO enrichment analysis of putative target genes. The top eight functions for biological process, cellular component, and molecular function. BP, CC, and MF had direct correlation between cell cycle, cell apoptosis, and the ingredients of SLC.

formed pi-sulfur interactions with the cyclic ring. Pro153 and Pro222 formed pi-alkyl interactions with the cyclic ring, which shows the potential hydrophobic interactions. Glu221 formed amide-pi stacked interactions with the cyclic ring. The molecular docking results indicated that there was stronger binding activity of the SLC's active ingredients with the primary targets of GC, which may imply that the SLC regulates the expression of the main pathways by combining with key targets. Besides, the results of binging energy between main ingredients such as stigmasterol, matrine, and main targets such as AKT1, TP53, CASP3, and EGFR also suggested that the main ingredients can control cell apoptosis and cell cycle to reduce the content of cancer cells The molecular docking results are shown in Table 3.

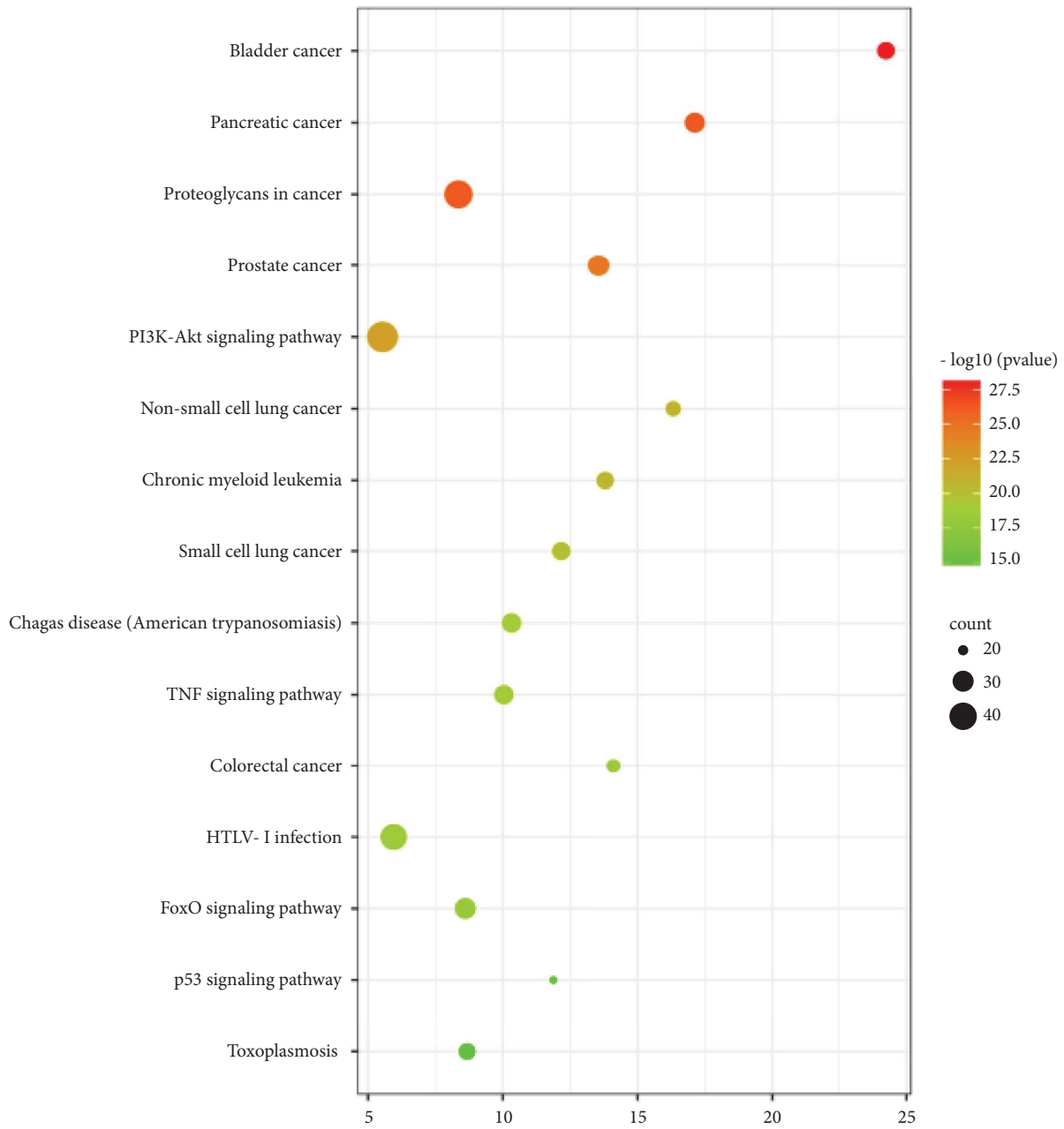


Figure 6: KEGG pathway enrichment analysis of putative target genes. There were twelve KEGG pathways with a significant P value <0.001. The targets of SLC had direct or indirect correlation with the KEGG pathways which correlated well with cancer. Thus, the ingredients of SLC could regulate these pathways such as TNF signaling pathway and PI3K-Akt signaling pathway to treatment.

3.5. Experimental Analysis

3.5.1. MTT Analysis. The SLC's antitumor effect on GC was confirmed by MTT assay, as shown in Figure 8. The results show that there was excellent effectiveness by the mid and high concentrations of the SLC in killing HGC-27 undifferentiated gastric cancer cells. The highest inhibition rate was up to 79.57% with different concentrations of the SLC solution (4 g/L) in a constant temperature incubator at 37°C for 48 h. Low cell viability was aided by the variety of ingredients, and the problem of drug resistance was solved. Therefore, the results of MTT analysis corroborate well with the conclusions of molecular docking. The ingredients of SLC such as quercetin, ursolic acid, stigmasterol, and matrine combined with key targets to prohibit cell apoptosis.

3.5.2. Investigation of Apoptosis and Cell Cycle through Flow Cytometry. To investigate the effect of the SLC combination on the cell cycle distribution, flow cytometry was used, as shown in Figure 9. The results collectively show that the SLC exerts a mild effect on the cell cycle in HGC-27 cells. Cells

Table 3: The molecular docking results of 4 key targets with 10 potential ingredients.

Target (PDB ID)	Ligand	Binding energy (kcal/mol)
AKT1 (6hhf)	Quercetin	−9.2
AKT1 (6hhf)	Ursolic acid	−8.9
AKT1 (6hhf)	Luteolin	−9.6
AKT1 (6hhf)	Formononetin	−9.3
AKT1 (6hhf)	Kaempferol	−9.2
AKT1 (6hhf)	beta-Sitosterol	−10.4
AKT1 (6hhf)	Wighteone	−10.0
AKT1 (6hhf)	Wogonin	−9.4
AKT1 (6hhf)	Stigmasterol	−10.7
AKT1 (6hhf)	Matrine	−8.3
TP53 (5g6o)	Quercetin	−7.9
TP53 (5g6o)	Ursolic acid	−7.3
TP53 (5g6o)	Luteolin	−8.2
TP53 (5g6o)	Formononetin	−8.1
TP53 (5g6o)	Kaempferol	−7.1
TP53 (5g6o)	beta-Sitosterol	−7.0
TP53 (5g6o)	Wighteone	−8.1
TP53 (5g6o)	Wogonin	−7.9
TP53 (5g6o)	Stigmasterol	−7.2
TP53 (5g6o)	Matrine	−8.0
CASP3 (3gjr)	Quercetin	−6.8
CASP3 (3gjr)	Ursolic acid	−8.7
CASP3 (3gjr)	Luteolin	−7.1
CASP3 (3gjr)	Formononetin	−7.3
CASP3 (3gjr)	Kaempferol	−6.7
CASP3 (3gjr)	beta-Sitosterol	−7.8
CASP3 (3gjr)	Wighteone	−7.6
CASP3 (3gjr)	Wogonin	−7.2
CASP3 (3gjr)	Stigmasterol	−7.8
CASP3 (3gjr)	Matrine	−8.1
EGFR (3lzb)	Quercetin	−8.0
EGFR (3lzb)	Ursolic acid	−6.7
EGFR (3lzb)	Luteolin	−8.0
EGFR (3lzb)	Formononetin	−7.6
EGFR (3lzb)	Kaempferol	−8.1
EGFR (3lzb)	beta-Sitosterol	−8.0
EGFR (3lzb)	Wighteone	−8.4
EGFR (3lzb)	Wogonin	−8.0
EGFR (3lzb)	Stigmasterol	−8.7
EGFR (3lzb)	Matrine	−8.5

were blocked in S phase at medium SLC concentrations and blocked in G1 phase at high SLC concentrations. The results of the flow cytometry assay proved that the main mechanism of action was regulation of apoptosis instead of decreasing cell proliferation. The results of flow cytometry evidenced the predictions of network pharmacology and molecular docking. SLC regulated cell cycle to promote apoptosis of HGC-27 cells.

3.5.3. Western Blotting. To confirm the results of network pharmacology, western blotting was used, as shown in Figure 10. We inferred that the SLC enhances proapoptotic gene expression, such as that of caspase-3 and TP53, to promote apoptosis. This result confirmed the potential targets studied from network pharmacology and molecular docking. We also verified the results of network pharmacology research. However, the inhibition of Akt and phosphorylated Akt was insufficient. We speculated that the reason for this may be a lack of effect on the cell cycle by the SLC. Quercetin, ursolic acid, stigmasterol, and matrine played very important role in the treatment. The results of western blotting justified that these main ingredients of SLC promote cell apoptosis to treat GC.

4. Discussion

Natural bioactive compounds are widely used to treat various diseases, especially cancers [23]. Natural products with potential for gastric cancer treatment have received much attention [24]. The possible mechanisms responsible for the effect of the SLCs in the treatment of advanced gastric cancer were proposed in this study. The main natural bioactive compounds of SLCs were identified by HPLC, and network pharmacology was used to identify the main potential active ingredients and targets [25]. SLCs may fortify

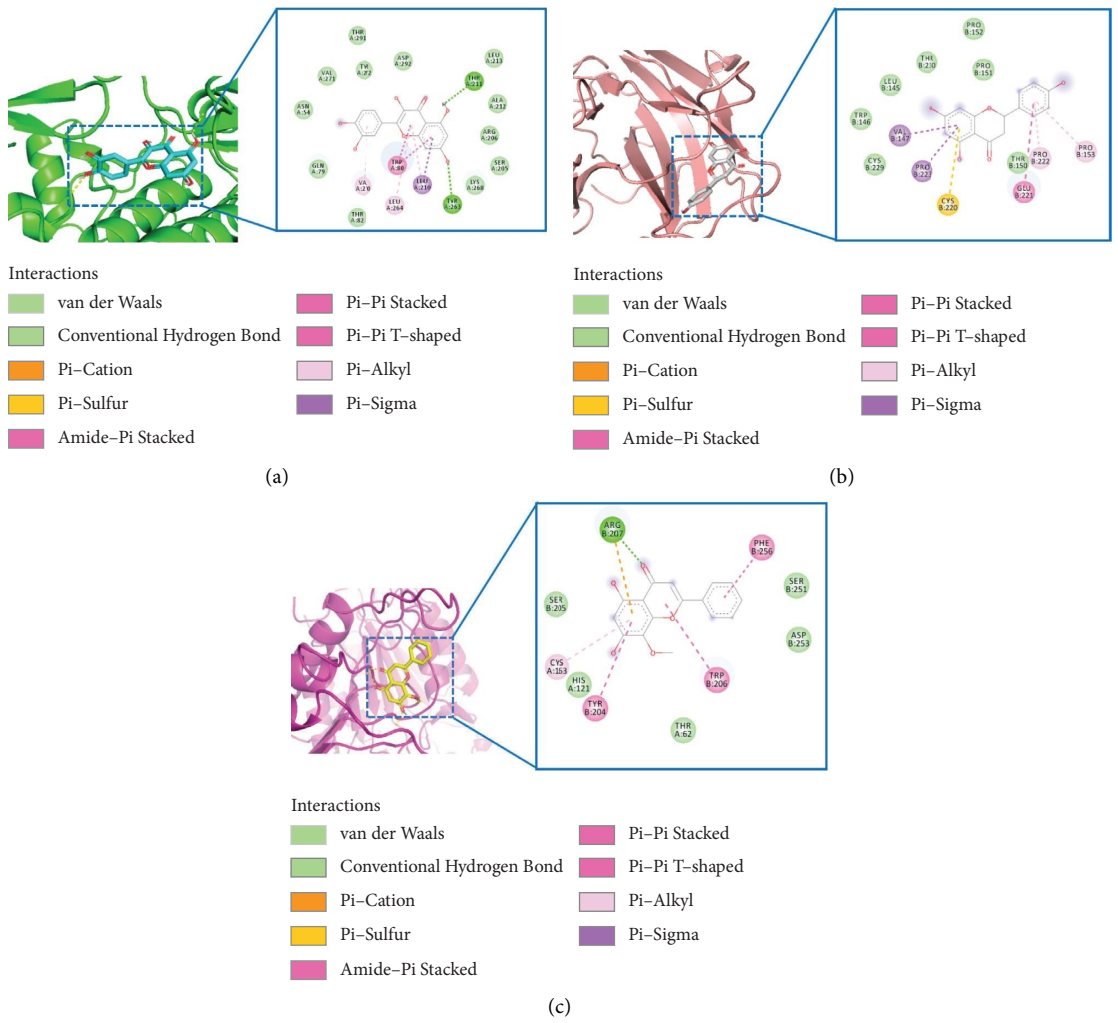


Figure 7: The molecular docking analysis of (a) AKT1 binding with quercetin, (b) TP53 binding with wogonin, and (c) CASP3 binding with (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one. Hydrogen bonds, pi-pi stacked, pi-sigma, and van der Waals forces contributed to the interaction between the targets and the ingredients.

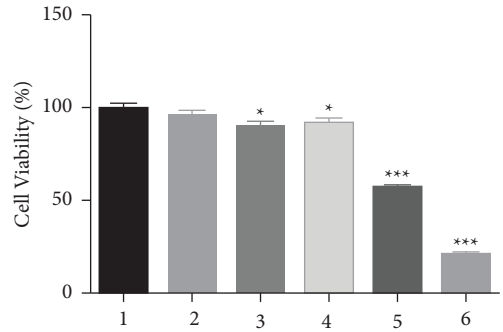


Figure 8: MTT assay. From left to right, the SLC concentrations were 0, 0.2, 0.5, 1, 2, and 4 g/L in turn. Each group was treated with the SLC for 48 h ($^{*}P < 0.1$ and $^{***}P < 0.001$). The cell viability of group 6 reached a plateau at ~20%.

the p53 signaling pathway and inhibit the PI3K/AKT signaling pathway to promote gastric cancer cell apoptosis. The p53 and PI3K/AKT signaling pathways play an important role in the apoptotic signaling circuitry [25, 26]. GO and KEGG enrichment analysis revealed that TP53, caspase-3, and AKT1 might be the key targets [27]. Network pharmacology study pointed out the relationship between ingredients of SLC such as quercetin, matrine, ursolic acid, and stigmasterol and key targets such as AKT1, EGFR, CASP3, and TP53, as shown in Figures 5 and 6. The main ingredients

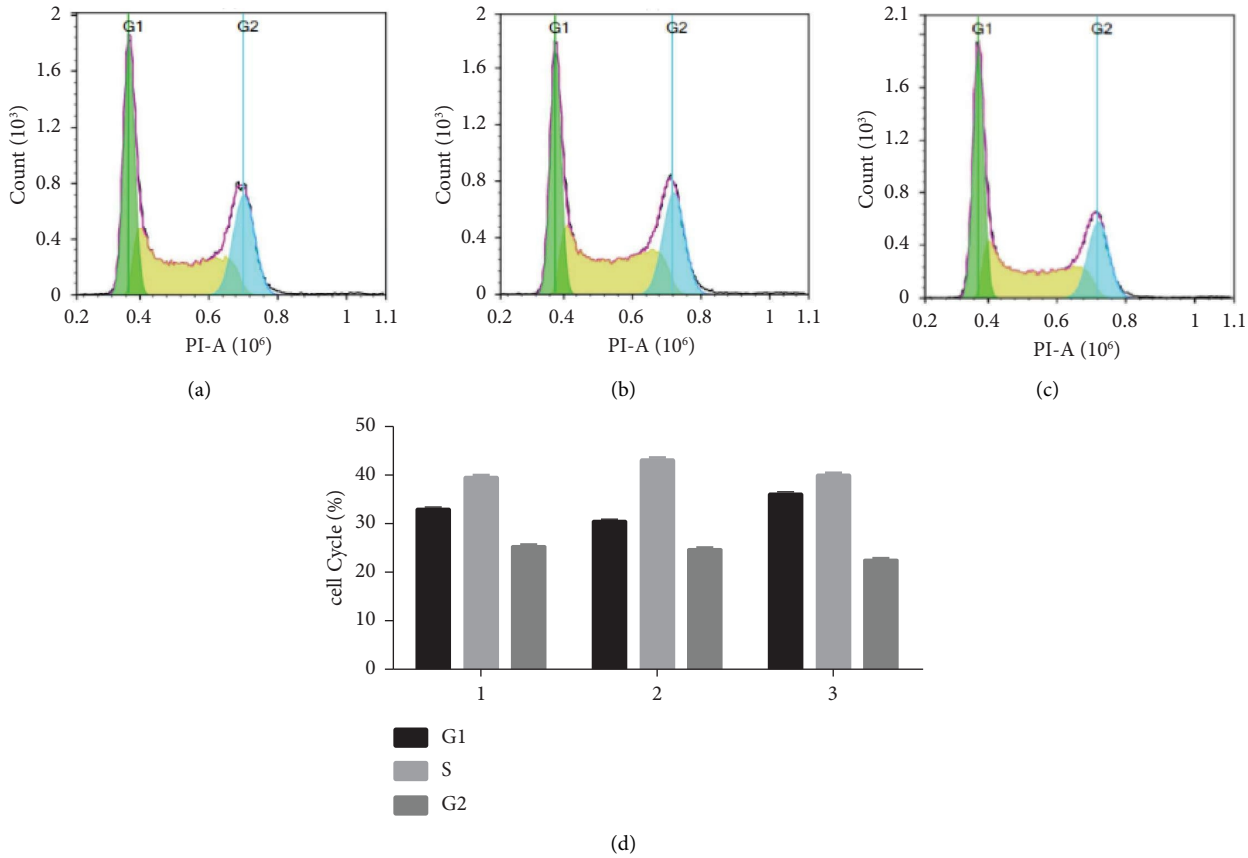


Figure 9: Flow cytometry analysis of HGC-27 cells. From left to right, the SLC concentrations were 0, 2, and 4 g/L. Each group was treated with SLCs for 48 h. HGC-27 cells were arrested in G1/S phase because of ingredients of SLCs.

of SLC might promote cell apoptosis and regulate cell cycle for GC therapy, and quercetin, matrine, and stigmasterol might be the main active natural bioactive compounds. In addition, numerous studies have already identified that these ingredients are potential anticancer agents and natural bioactive compounds can be expected to address clinical drug resistance [28–30], and the accuracy of the prediction was confirmed.

Furthermore, molecular docking revealed the results of ingredients binding to targets. There were excellent docking results for quercetin, beta-sitosterol, ursolic acid, matrine, and stigmasterol. These main ingredients were docked with main targets such as AKT1, CASP3, TP53, and EGFR. The results of molecular docking confirmed that SLC's ingredients could combine with these key targets to control PI3K-Akt signaling pathway, p53 signaling pathway, and TNF signaling pathway. This matched the network pharmacology study's interpretation and offered a cell experimental basis for the use of key targets as therapeutic targets. This adds further evidence that the natural bioactive compounds in SLC have promising effect on GC.

Moreover, the results of cell experimental were in a good agreement with the network pharmacology and molecular docking results. The MTT assay and flow cytometry also confirmed that SLCs can prompt the apoptosis and inhibit proliferation of HGC-27 cells. Although the SLC exerted a mild effect on the cell cycle in HGC-27 cells, cell viability decreased to 11.43% after HGC-27 cells were incubated with high concentrations of the SLC. This matches the results of previous studies and pointed out that the ingredients of SLC could promote cell apoptosis and control cell cycle to reduce the viability of HGC-27 cells in vitro. Western blotting confirmed that the SLC upregulated the protein expression of TP53 and caspase-3 protein, which consequently promoted cell apoptosis [31–34]. This confirmed that the ingredients of SLC regulated the expression of the TNF signaling pathway and p53 signaling pathway and led to cell apoptosis. The accuracy of network pharmacology and molecular docking prediction results must be verified by experimental assay.

Nevertheless, we systematically demonstrated the potential mechanism of the SLCs and confirmed that the SLC's action in the treatment of advanced gastric cancer is the regulation of the p53 signaling pathway overexpression. The main active natural bioactive compounds might be quercetin, matrine, ursolic acid, and stigmasterol. As a TCM that is rich in plant-derived compounds, there is great potential for the SLC to play a more significant role in the treatment of advanced gastric cancer. Moreover, the natural bioactive compounds such as quercetin, matrine, and ursolic acid are

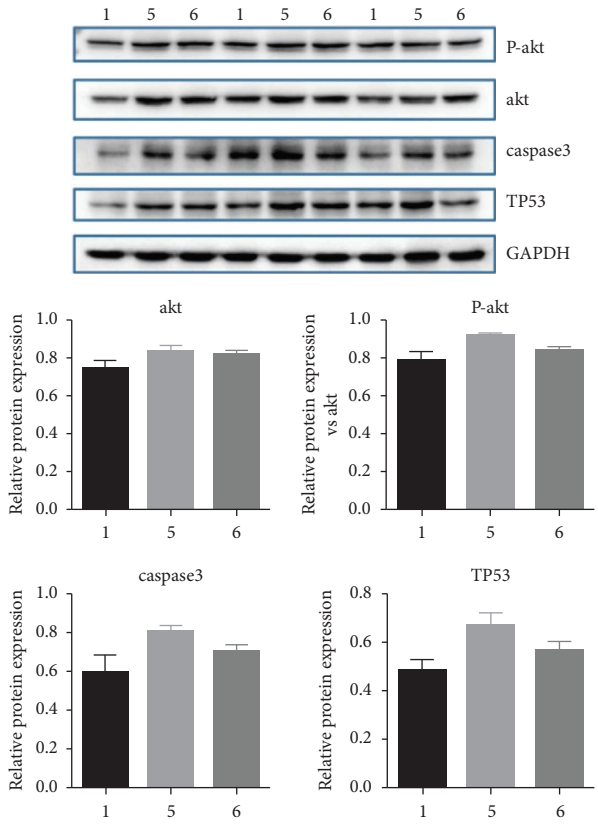


Figure 10: Western blot assay of phosphorylated Akt, Akt, CASP3, and TP53. From left to right, the SLC concentrations were 0, 2, and 4 g/L. Caspase3 and TP53 of HGC-27 cells were overexpressed after treated with SLC concentrations.

useful in the therapy of gastric cancer. Natural products are considered a source for bioactive compounds and have potential for developing some novel therapeutic agents.

Data Availability

All the data and materials used in the current study are available from the corresponding author upon reasonable request.

Disclosure

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Figure 1: the drug/target/pathway network. Supplementary Figure 2: the raw data of MTT assay. Supplementary PDF1: the raw data of flow cytometry W1. Supplementary PDF2: the raw data of flow cytometry W2. Supplementary PDF3: the raw data of flow cytometry W3. Supplementary PDF4: the raw data of western blot. (*Supplementary Materials*)

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