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Molecular Mechanism of *Senecio scandens* Buch.-Ham. in Regulating Hepatic Carcinoma Based on Bioinformatics and Network Pharmacology

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Abstract:

Objective: To investigate the molecular mechanism of *Senecio scandens* Buch.-Ham. in regulating hepatocellular carcinoma through bioinformatics and network pharmacology techniques and methodologies. *Methods:* The active components and targets of *Senecio scandens* Buch.-Ham. were identified using the systematic pharmacology database and analysis platform for traditional Chinese medicine, the traditional Chinese medicine database of Taiwan region, and the bioinformatics database for molecular mechanisms of traditional Chinese medicine. Datasets GSE57957, GSE60502, and GSE84402 were downloaded from public gene expression databases, and differentially expressed genes (DEGs) were analyzed using the GEO2R online tool. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed using the DAVID database. Protein interactions and the "active component-target-disease" network were constructed using the STRING database and Cytoscape 3.7 software to identify key genes. Expression differences and prognosis were analyzed using the GEPIA database. AutoDock 4.6 software was utilized for the docking verification of candidate targets. *Results:* A total of eight active components, including 2-furancarboxylic acid, 6-hydroxy-2-(2-phenylethyl)chromone, chrysanthemaxanthin, chrysanthemic acid, flavoxanthin, hydroquinone, p-hydroxyacetophenone, and seneciphylline, were identified with 321 targets. The three datasets (GSE57957, GSE60502, and GSE84402) revealed 245 common DEGs. In the "active component-target-disease" network, six components were involved in regulating hepatic carcinoma, with 17 common targets. GO biological processes involved ethanol oxidation, redox reactions, the cyclooxygenase P450 pathway, and fatty acid long-chain metabolism. KEGG pathways involved the fatty acid degradation pathway, chemical carcinogenesis pathway, metabolic pathway, retinol pathway, and cytochrome P450 metabolism pathway. Key targets in the protein-protein interaction network were *CYP1A1*, *PTGS2*, *ESR1*, and *NQO1*. *ESR1* and *NQO1* showed significant differences and were associated with poor prognosis. Molecular docking verification indicated a close binding of *NQO1* to the active ingredient. *Conclusion: Senecio scandens* Buch.-Ham. may exert an auxiliary anti-hepatocellular carcinoma effect by regulating metabolic pathways in hepatocellular carcinoma cells.

Keywords:

Hepatic carcinoma
Senecio scandens Buch.-Ham.
Bioinformatics
Network pharmacology
Molecular mechanism

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1. Introduction

The incidence of liver cancer ranks sixth among all malignancies, and its mortality rate ranks fourth [1], with a higher incidence in males than in females. In China, the incidence of liver cancer is gradually increasing. Liver cancer not only threatens the lives of patients but also imposes a heavy economic burden on the country and families. Clinical treatment includes surgical resection, radiotherapy, radiofrequency ablation, allograft transplantation, and chemotherapy, among which drug therapy plays a crucial role. Traditional Chinese medicine (TCM) can not only reduce the toxic and side effects of radiotherapy and chemotherapy in the adjuvant treatment of liver cancer but also improve the sensitivity of cancer cells to drugs. In TCM, liver cancer is classified into categories such as "jaundice," "liver accumulation," and "rib pain," and its syndromes are differentiated as liver depression and spleen deficiency, dampness-heat in the liver and gallbladder, liver heat and blood stasis, and liver and kidney yin deficiency. Different syndromes require different medications [2]. For the dampness-heat syndrome in the liver and gallbladder, TCM adopts herbs that clear heat and promote diuresis, cool blood, and detoxify. *Senecio scandens* Buch.-Ham., a plant belonging to the *Senecio* genus in the Asteraceae family, has a bitter taste and cold properties. It has the effects of clearing heat and detoxifying, improving eyesight, and promoting diuresis. Its pharmacological effects include antibacterial, anti-inflammatory, antitumor, and antioxidant activities [3]. However, the active components and targets of *Senecio scandens* Buch.-Ham. involved in the prevention and treatment of liver cancer are rarely reported due to its complex composition. This study aims to elucidate the active components, targets, and underlying mechanisms of *Senecio scandens* Buch.-Ham. in regulating liver cancer using bioinformatics and network pharmacology techniques and methods, providing a theoretical basis for research on adjuvant antitumor medications (**Figure 1**).

2. Materials and methods

2.1. Active ingredient screening and target prediction

Compounds from *Senecio scandens* Buch.-Ham. were retrieved from the TCM Database@Taiwan (http://tcm.cmu.edu.tw) and mapped to the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (https://tcmsp-e.com/tcmsp.php) to obtain information on compounds and their targets. Additionally, the compound "QIANLIGUANG" was searched in the Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) (http://bionet.ncpsb.org.cn/batman-tcm/) with parameters set to a binding score (Score) > 20 and significant difference $P < 0.05$ to acquire compound-target data. The data from these three databases were then merged and duplicates were removed.

2.2. Differential expression genes (DEGs) screening and construction of "active ingredient-target-disease" network

The Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) was searched using the keyword "liver cancer," yielding three liver cancer-related microarray datasets: GSE57957, GSE60502, and GSE84402. GSE57957: From the Functional Genomics Laboratory of Singapore, it includes 59 tumor tissue samples and 59 adjacent non-tumor samples GSE60502: From the Koo Foundation SYS Cancer Center in Taiwan region, it consists of 18 tumor tissue samples and 18 adjacent non-tumor samples GSE84402: From the Shanghai Cancer Institute, it contains 18 tumor tissue samples and 18 non-tumor samples. The datasets were

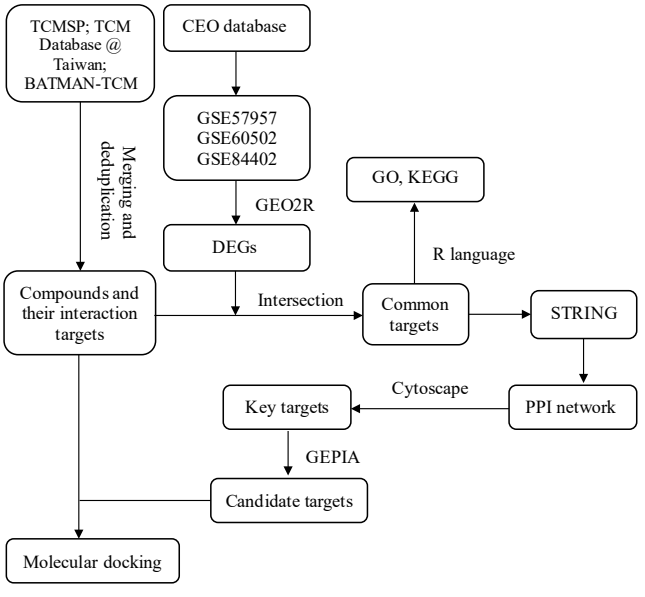


Figure 1. Flow chart of the technical route of this study

analyzed using the GEO2R online platform, applying screening criteria of $P < 0.05$ and $|logFC| \geq 1$ to identify differentially expressed genes (DEGs). A Venn diagram was generated using the ggplot2 package in R to find the intersection of DEGs among the datasets, yielding common DEGs. A compound-target-disease network was constructed using Cytoscape 3.7 software to identify active components and their targets.

2.3. Construction of protein interaction network and screening of key targets

The common DEGs were uploaded to the STRING database (https://cn.string-db.org/) with the species set to "*Homo sapiens*" and the combined score threshold set to Score > 0.4, while other parameters were left at their default settings. This process constructed a protein-protein interaction (PPI) network, which was visualized using Cytoscape 3.7 software. The PPI network was analyzed using the "CytoHubba" and "MCODE" plugins in Cytoscape to predict key targets within the network.

2.4. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The common DEGs were uploaded to the DAVID database (https://david.ncifcrf.gov/), "official gene symbol" was selected for "select identifier," "*Homo sapiens*" was chosen for species, and "gene list" for "list type" to obtain the biological processes, components, molecular functions, and signaling pathways involved in the targets.

2.5. Expression and survival analysis of key targets in liver cancer

The single gene analysis section was selected in the GEPIA database (http://gepia.cancer-pku.cn/), the key targets were entered in the "enter gene name" search bar to obtain the expression of targets in normal individuals and liver cancer patients, as well as their impact on the overall survival rate of liver cancer patients. Genes with significant differences and poor prognosis were selected as candidate targets.

2.6. Molecular docking verification of active ingredients and candidate targets

The 2D structural formulas of active ingredients were obtained from the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/), saved as SDF files, and converted to mol2 format using OpenBabel software. Candidate target ID numbers were searched in the UniProt database (https://www.uniprot.org/), entering the ID numbers into the RCSB database (https://www.rcsb.org/) to obtain the PDB ID and original ligand information of the targets, and using AutoDock 4.6 software to perform molecular docking simulations on the candidate targets and active ingredients.

2.7. Statistical analysis

The expression results of key targets obtained from the GEPIA database were displayed as *P*-values, with $P < 0.01$ considered statistically significant. For survival analysis, $P < 0.05$ was considered statistically significant, and survival differences were compared using the Log-rank test. Spearman's correlation coefficient was used to evaluate the relationship between gene expressions of key targets.

3. Results

3.1. Active ingredients and targets of Senecio scandens

By searching the TCMSP, TCM Database@Taiwan, and BATMAN-TCM databases, a total of eight active ingredients were obtained, with M1 being common to both TCMSP and TCM Database@Taiwan, and M8 originating from BATMAN-TCM. After removing duplicates, a total of 321 targets for these compounds were identified. **Table 1** shows basic information on the active ingredients of *Senecio scandens*.

3.2. General information

The GSE57957 and GSE60502 datasets did not record clinical information, while the GSE84402 dataset documented basic information for 28 samples (14 liver cancer patients and 14 normal individuals), including age, gender, source, and solid tumor size (**Table 2**).

Table 1. Basic information on active ingredients of *Senecio scandens*

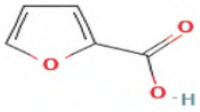
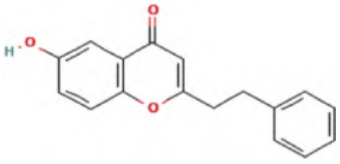
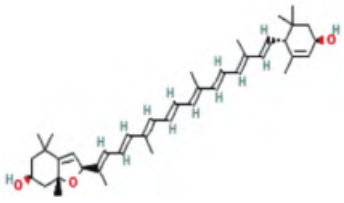
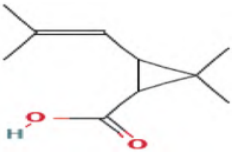
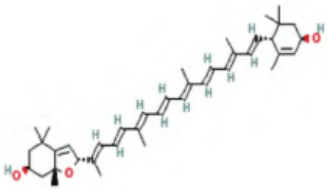
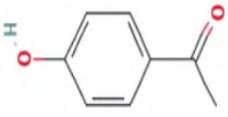
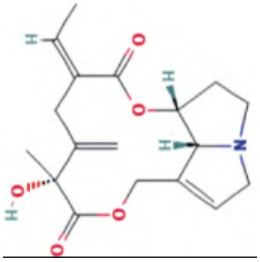
Code	Active ingredient name	Chemical structure	Molecular formula	Molecular weight
M1	2-furoic acid		$C_5H_4O_3$	112.083
M2	6-hydroxy-2-(2-phenylethyl) chromone		$C_{17}H_{14}O_3$	266.291
M3	Chrysanthemin		$C_{40}H_{56}O_3$	584.871
M4	Chrysanthemic acid		$C_{10}H_{16}O_2$	168.233
M5	Flavoxanthin		$C_{40}H_{56}O_3$	584.871
M6	1,4-benzenediol		$C_6H_6O_2$	69.280
M7	4-Hydroxyacetophenone		$C_8H_8O_2$	136.148
M8	Seneciphylline		$C_{18}H_{23}NO_5$	333.380

Table 2. General information of subjects

Number	Type	Gender	Age (years)	Hepatitis B virus surface antigen	Tissue source	Tumor Size (mm)
Liver cancer group						
CSM2233090	Hepatocellular carcinoma tissue	Male	40	Positive	Liver	9×8×8
CSM2233112	Hepatocellular carcinoma tissue	Male	-	Positive	Liver	-
CSM2233110	Hepatocellular carcinoma tissue	Male	50	Positive	Liver	-
CSM2233108	Hepatocellular carcinoma tissue	Male	54	Positive	Liver	11×9×7
CSM2233088	Hepatocellular carcinoma tissue	Male	35	Positive	Liver	5×4×4
CSM2233106	Hepatocellular carcinoma tissue	Female	35	Positive	Liver	11×10×4.5
CSM2233104	Hepatocellular carcinoma tissue	Female	44	Positive	Liver	9×10×7
CSM2233102	Hepatocellular carcinoma tissue	Female	40	Positive	Liver	20×15×18
CSM2233100	Hepatocellular carcinoma tissue	Female	67	Positive	Liver	Left lateral lobe 10×12, right lobe 3×2
CSM2233098	Hepatocellular carcinoma tissue	Female	43	Positive	Liver	3×3×3
CSM2233096	Hepatocellular carcinoma tissue	Male	45	Positive	Liver	3×2
CSM2233094	Hepatocellular carcinoma tissue	Male	50	Positive	Liver	7×9×7
CSM2233092	Hepatocellular carcinoma tissue	Male	54	Positive	Liver	＞ 10
CSM2233086	Hepatocellular carcinoma tissue	Male	63	Positive	Liver	7.3×6.6×6.5
Control group						
CSM2233091	Non-cancerous tissue	Male	40	Positive	Liver	-
CSM2233089	Non-cancerous tissue	Male	35	Positive	Liver	-
CSM2233113	Non-cancerous tissue	Male	-	Positive	Liver	-
CSM2233111	Non-cancerous tissue	Male	50	Positive	Liver	-
CSM2233109	Non-cancerous tissue	Male	54	Positive	Liver	-
CSM2233107	Non-cancerous tissue	Female	35	Positive	Liver	-
CSM2233105	Non-cancerous tissue	Female	44	Positive	Liver	-
CSM2233103	Non-cancerous tissue	Female	40	Positive	Liver	-
CSM2233101	Non-cancerous tissue	Female	67	Positive	Liver	-
CSM2233099	Non-cancerous tissue	Male	43	Positive	Liver	-
CSM2233087	Non-cancerous tissue	Male	63	Positive	Liver	-
CSM2233097	Non-cancerous tissue	Male	45	Positive	Liver	-
CSM2233095	Non-cancerous tissue	Male	50	Positive	Liver	-
CSM2233093	Non-cancerous tissue	Male	54	Positive	Liver	-

3.3. DEGs screening and volcano plot drawing

The online analysis tool GEO2R was used to analyze the GSE57957, GSE60502, and GSE84402 microarray datasets, and 417, 1607, and 3549 DEGs were screened out, respectively. Using the R language to find the intersection, 245 common DEGs were obtained, and a volcano plot was drawn using the “ggplot2” package (**Figure 2**).

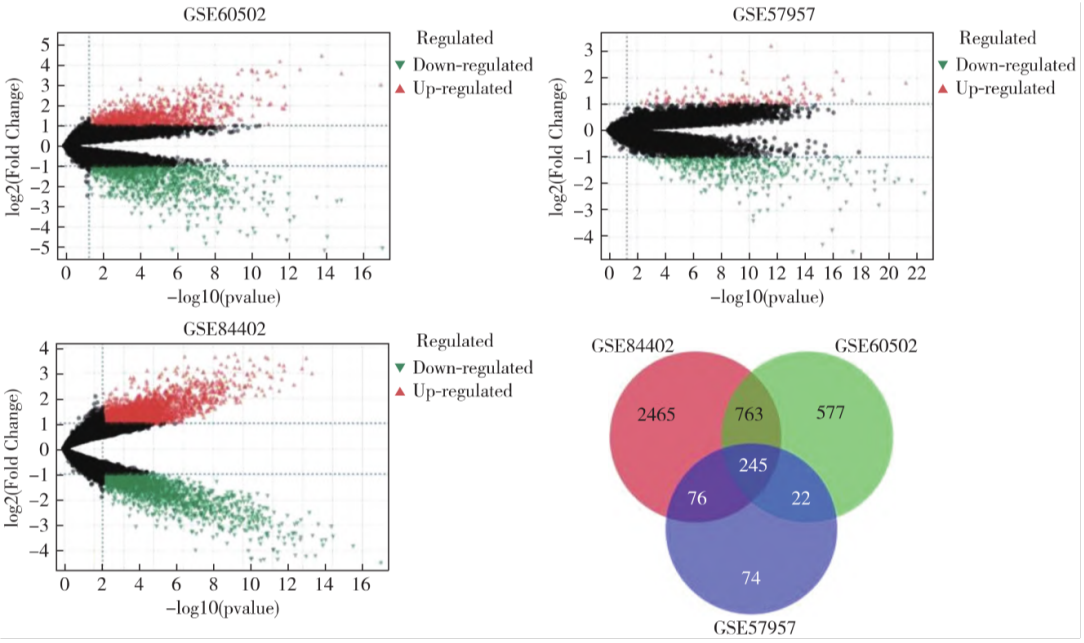


Figure 2. Volcano plot and Venn diagram of DEGs. Note: Red triangles represent upregulation, green triangles represent downregulation, and black dots represent non-significant fold differences

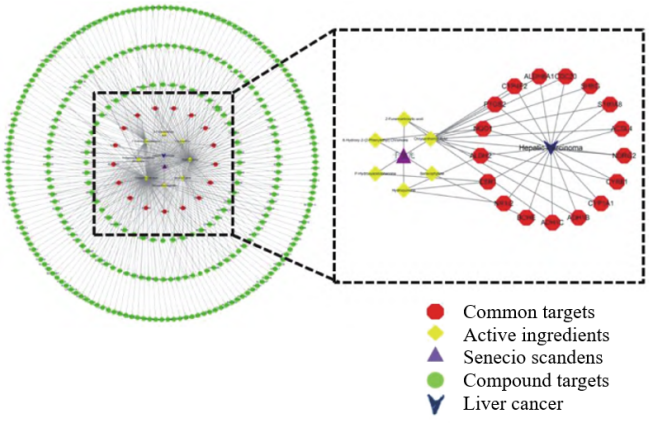


Figure 3. "Active ingredient-target-disease" network

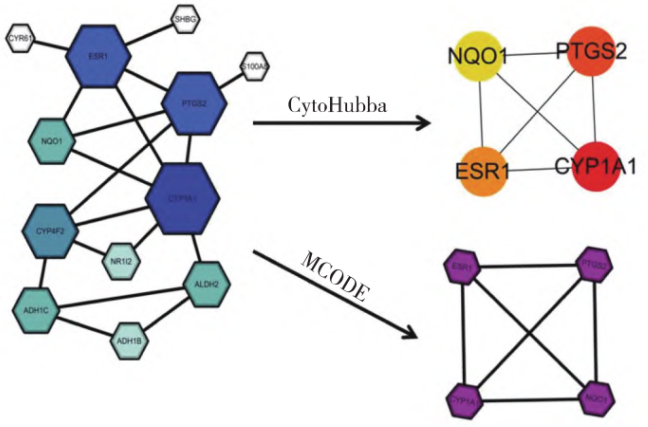


Figure 4. Protein-protein interaction network and key targets

3.4. Construction of the "active ingredient-target-disease" network and screening of common targets

The "active ingredient-target-disease" network includes eight active ingredients and 321 targets. There are six components involved in the regulation of liver cancer, and 17 common targets, which are *ESR1*, *ALDH2*, *NDIG2*, *NQO1*, *PTGS2*, *CYP4F2*, *ALDH8A1*, *CDC20*, *SHBG*, *S100A8*, *ACSL4*, *CYR61*, *CYP1A1*, *ADH1B*, *ADH1*, *CBCHE*, and *NR1I2* (**Figure 3**).

3.5. Construction of protein-protein interaction (PPI) network and screening of key targets

The 17 common targets were imported into the STRING data platform to obtain PPI network data in tsv format. Visual analysis was performed using Cytoscape 3.7 software (excluding isolated targets). The network was analyzed using two algorithms, "CytoHubba" and "MCODE," to obtain key targets such as *CYP1A1*, *PTGS2*, *ESR1*, and *NQO1* (**Figure 4**).

3.6. GO and KEGG pathway enrichment analysis

GO functional enrichment covers biological processes (BP), cellular components (CC), and molecular functions (MF) (**Figure 5**). BP involves ethanol oxidation, oxidation-reduction reactions, estradiol reactions, responses to lipopolysaccharide, the cytochrome P450

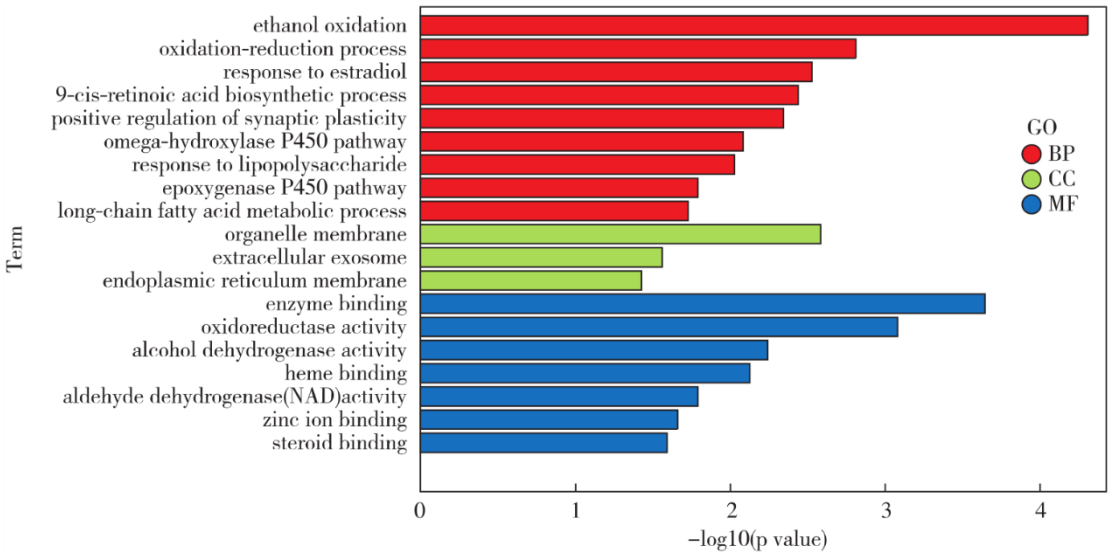


Figure 5. GO functional analysis

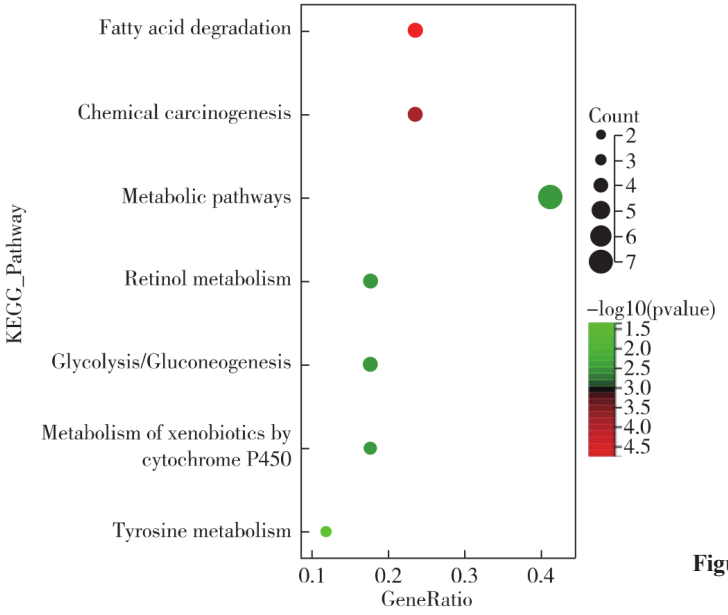


Figure 6. KEGG pathway enrichment analysis of common targets

pathway, and long-chain fatty acid metabolism; CC relates to organelle membranes, extracellular exosomes, and endoplasmic reticulum membranes; MF pertains to enzyme binding, oxidoreductase activity, alcohol dehydrogenase activity, heme binding, and aldehyde dehydrogenase activity. KEGG enrichment identified seven pathway categories, namely fatty acid degradation, chemical carcinogenesis, metabolic pathways, retinol metabolism, and the cytochrome P450 metabolic pathway for xenobiotics (**Figure 6**).

3.7. Significant differences and survival analysis of key targets

The expression of *CYP1A1*, *ESR1*, and *NQO1* in liver cancer tissues showed significant differences compared to the control group ($P < 0.01$). Survival analysis can identify genes most significantly related to patient survival. Genes with significant differences were analyzed for overall survival (OS), and *ESR1* and *NQO1* were found to be associated with OS ($P < 0.05$). High expression of *NQO1* and low expression of *ESR1* are both associated with poor prognosis in liver cancer patients. It is speculated that *ESR1* and *NQO1* may be key targets for the regulation of liver cancer by *Senecio scandens* and can be used as candidate targets for further validation (**Figure 7**).

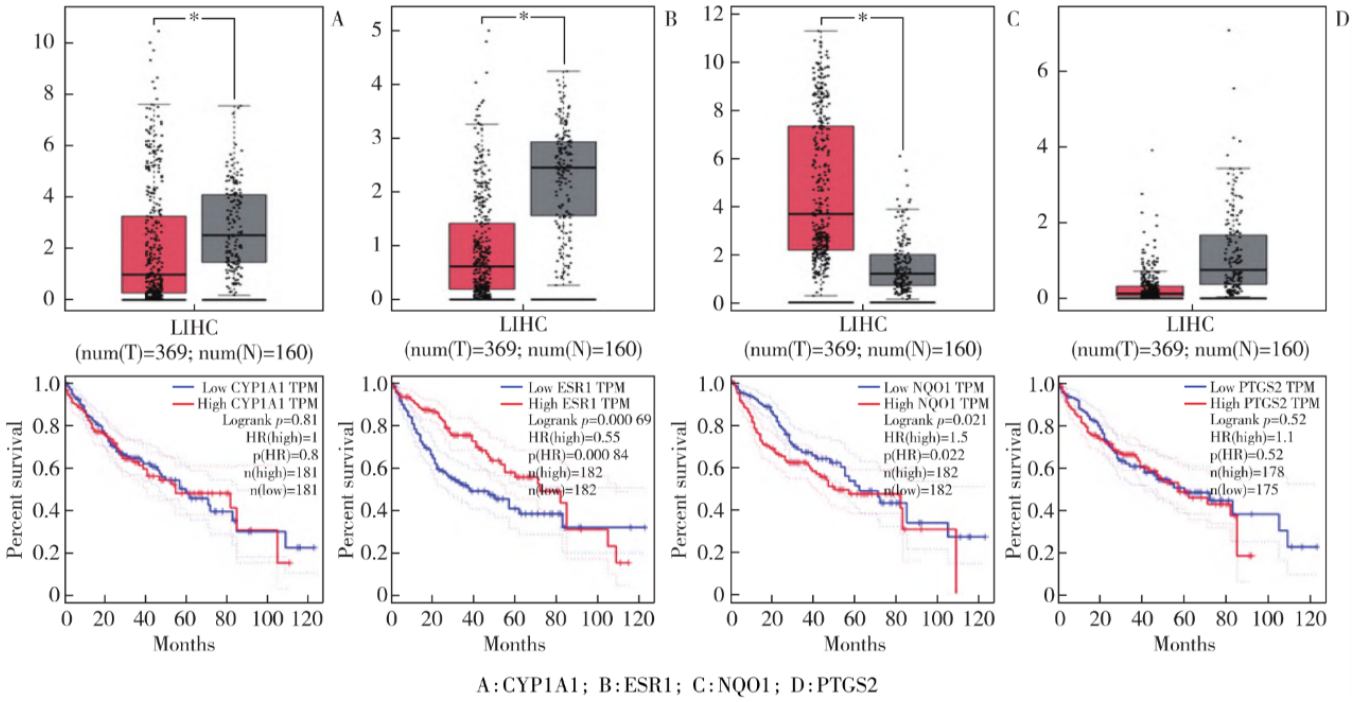


Figure 7. Expression of *CYP1A1*, *ESR1*, *NQO1*, and *PTGS2* genes in liver cancer tissues and their relationship with prognosis in the GEPIA database

Table 3. Molecular docking information of active ingredients with candidate targets

Target name	PDB ID	Original ligand	3D coordinates of active site	Affinity of original ligand	Affinity of active ingredient	
ESR1	2IOK	IOK	x = 26.979; y = 26.979; z = 40.288	-5.3	4-hydroxyacetophenone -3.39	1,4-benzenediol -3.39
NQO1	5EA2	FAD	x = -6.089; y = -1.426; z = -12.998	-0.85	6-hydroxy-2-(2-phenylethyl)chromone -5.07	

Note: Affinity unit: kcal/mol.

3.8. Molecular docking validation

The PDB IDs for *ESR1* and *NQO1* were obtained from the RCSB database, which are 2IOK and 5EA2, respectively. Active ingredients interacting with these targets were selected, and docking validation was performed using AutoDock version 4.6 software. The results showed that 4-hydroxyacetophenone and 1,4-benzenediol bind less strongly to 2IOK than the original ligand IOK. Conversely, 6-hydroxy-2-(2-phenylethyl)chromone binds much stronger to 5EA2 than the original ligand FAD, suggesting that 6-hydroxy-2-(2-phenylethyl)chromone could be a candidate molecule for further investigation (**Table 3** and **Figure 8**).

4. Discussion and conclusion

Senecio scandens is a perennial herb belonging to the Asteraceae family. It is often used to treat diseases such as hepatitis, upper respiratory tract infections, cholecystitis, trauma, stagnation and swelling, and ulcerations due to its effects of clearing heat and detoxifying, promoting blood circulation and improving vision, reducing swelling and relieving pain, and dispelling wind and eliminating dampness [4-6]. Traditional Chinese medicine has a unique effect on preventing and improving cancer control and needs to be used in combination with other drugs. This study combined bioinformatics technology and network pharmacology methods to explore the pharmacological

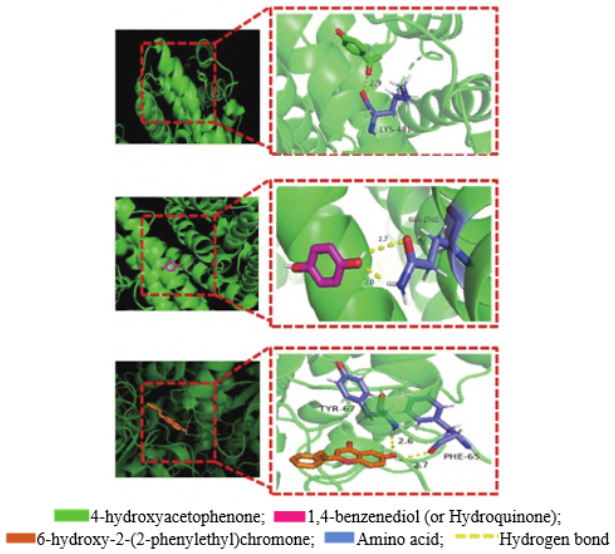


Figure 8. Molecular docking of active ingredients in *Senecio scandens* and candidate targets. 4-hydroxyacetophenone docks with the LYS-481 residue (hydrogen bond length 2.9 Å); 1,4-benzenediol docks with the GLN-1502 residue (hydrogen bond lengths are 2.7 Å and 2.0 Å respectively); 6-hydroxy-2-(2-phenylethyl)chromone docks with TYR-67 (hydrogen bond length is 2.6 Å) and PHE-65 (hydrogen bond length is 2.7 Å) respectively.

mechanism of *Senecio scandens* in regulating liver cancer. Eight active ingredients were identified in *Senecio scandens*, six of which are involved in regulating 17 liver cancer DEGs. These genes are mainly concentrated in fatty acid degradation, metabolic pathways, retinol metabolism pathways, and the CYP450 metabolic pathway for xenobiotics.

Through the analysis of the protein interaction network, key targets of the network were identified, namely *CYP1A1*, *PTGS2*, *ESR1*, and *NQO1*. *CYP1A1* is a sub-enzyme of the CYP1 enzyme system involved in drug metabolism, catalyzing the metabolism of aromatic amines, heterocyclic amines, benzophenones, and some carcinogens containing halogenated hydrocarbons. Changes in *CYP1A1* gene activity are closely related to the susceptibility of liver cancer [7,8]. *PTGS2*, also known as prostaglandin peroxidase synthase 2 (COX2), is associated with increased cell adhesion, phenotypic changes, inflammatory responses, and tumor angiogenesis [9]. *ESR1*, also known as estrogen receptor 1, is a nucleic acid receptor of the ligand-activated transcription factor family. It has the function of transcriptional regulation of proteins and can affect the expression and regulation of estrogen genes. *NQO1*, known as quinone oxidoreductase 1, is an important phase II antioxidant enzyme in the body. Using NADPH as the receptor, it catalyzes reduction reactions including reactive oxygen species and coenzyme Q. Under the action of this enzyme, quinones in the body are reduced to hydroquinones, reducing the production of oxygen free radicals from quinone conversion and lowering damage to normal tissues and organs from free radicals, thus maintaining the structural stability of mitochondria and other organelles [10]. It is speculated that the above key target genes are involved in the occurrence, development, and chemotherapy drug metabolism of liver cancer. Analysis of key target expression and survival using the GEPIA database showed that *ESR1* and *NQO1* are associated with poor prognosis in liver cancer. Molecular docking methods were used to verify the action of the active ingredients of *Senecio scandens* on the above key targets. The results showed that the binding of 6-hydroxy-2-(2-phenylethyl) chromone to *NQO1* was higher than that of the original ligand, suggesting that 6-hydroxy-2-(2-phenylethyl) chromone can be used as a candidate molecule for further study.

In recent years, network pharmacology research on liver cancer has been very extensive, but there are errors in the screening of active ingredients and target sites of traditional Chinese medicines, and there is a lack of clinical research sample support. This study adopted bioinformatics methods, incorporated clinical samples into the analysis, and conducted significant difference and survival analysis on predicted targets based on this, excluding false-positive interference. At the same time, to verify the degree of binding between active ingredients and targets, molecular docking verification was performed. However, this study only used literature indexing and chemical composition simulation screening, which may have ignored differences in active ingredient content and drug interactions. To clarify the research results, corresponding biological experiments need to be conducted on this basis.

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Disclosure statement

The authors declare no conflict of interest.

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