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Mechanism of action of polyphenols from *Erigeron breviscapus* on liver fibrosis

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Abstract This study employs combined network pharmacology and molecular docking approaches to investigate the potential mechanisms by which *Erigeron breviscapus* polyphenols inhibit liver fibrosis. Active compounds were identified through literature mining, with targets predicted using TCMSP, PubChem, SwissTarget, and SwissADME databases. Liver fibrosis-related targets were retrieved from GeneCards, OMIM, and TTD. Following rigorous screening, 12 bioactive polyphenolic compounds and 117 corresponding targets were identified, intersecting with 8,375 liver fibrosis targets to yield 67 common targets. Protein-protein interaction analysis revealed 80 key targets (e.g., EGFR, ESR1, PTGS2). GO and KEGG analyses indicated enrichment in 352 biological terms and 50 pathways, including chemical carcinogenesis receptor activation and steroid hormone biosynthesis. Molecular docking confirmed effective binding affinity between the top four compounds (by degree value) and their respective targets. In summary, the results of this study indicate that *Erigeron breviscapus* can inhibit the development of liver fibrosis and related diseases through multiple components, targets, and pathways. This study provides a solid theoretical basis for the research of *Erigeron breviscapus* in the field of anti liver fibrosis.

Keywords: *Erigeron breviscapus*; polyphenol; liver fibrosis; network pharmacology; molecular docking

1 Introduction

China bears a high global burden of liver diseases, with hepatitis B virus (HBV) infection exhibiting the world's highest incidence rate. Approximately 380,000 annual deaths in China are attributed to cirrhosis or hepatocellular carcinoma (HCC), accounting for over 50% of global HCC mortality [1]. Liver fibrosis constitutes a crucial and reversible stage in the progression towards liver cirrhosis. If left untreated, it can eventually progress to cirrhosis or HCC leading to death [2]. Due to the lack of treatment options for cirrhosis and HCC, there is an urgent need to find a new treatment method for liver fibrosis. The global market value of liver fibrosis drugs is expected to reach 126 million US dollars by 2025 [3]. However, there are currently no approved anti-liver fibrosis drugs on the market, and first-line drugs such as interferon have certain side effects. The advantages of traditional Chinese medicine in improving liver fibrosis symptoms, restoring liver function, improving in trahepatic blood circulation,

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reversing liver fibrosis and regulating immunity have led to a consensus between Chinese medicine and Western medicine. Therefore, finding highly efficient, low toxicity, and cost-effective liver fibrosis protectants from natural plants has become an urgent need in the field of health [4]. Natural plant polyphenols include mainly flavonoids, coumarins, phenolic acids, and styrene based pyranone compounds [5]. Natural plant polyphenols have emerged as a focal point of research for the prevention and adjuvant treatment of liver diseases, owing to their distinctive physiological activities and promising health advantages [6]. Therefore, it is crucial to explore natural plant polyphenol bioactive ingredients, drugs, and methods that can reverse liver fibrosis.

Erigeron breviscapus (Vant.) Hand-MazzIt is the dry whole plant of the asteraceae plant, *Erigeron breviscapus*, as a traditional Chinese herbal medicine, it has a cultivation history of approximately 1000 years in China. In traditional Chinese medicine, *Erigeron breviscapus* has been proven to dispel cold, relieve external symptoms, dispel wind and dampness, activate collaterals and relieve pain. Modern studies have shown that the flavonoids are among the main active ingredients extracted from the *Erigeron breviscapus*, and their pharmacological effects include anti-inflammatory, antioxidant, lipid-lowering, vasodilator, anti-platelet, and anti-coagulant [7]. In recent years, it has been proven to have significant protective effects on vascular diseases [8]. At the same time, it has significant therapeutic effects on cardiovascular and cerebrovascular diseases [9]. Therefore, over the years, research reports on breviscapine have mostly focused on its ability to treat hypertension, ischemic stroke, coronary heart disease, and angina pectoris. Advancements in science and technology have led to the confirmation, through extensive research, of various types of polyphenolic compounds extracted from *Erigeron breviscapus*. These compounds encompass quercetin, luteolin, baicalin, kaempferol, formononetin, scutellarin, as well as breviscapine, among others [10]. Recently, Liu Y et al. [11] conducted an evaluation of the impact of breviscapine on acute liver injury by establishing a mouse model of liver damage induced by CCl_4. Their findings indicate that breviscapine exerts a protective role by inhibiting inflammation and apoptosis in CCl_4-induced acute liver injury. Furthermore, Tian Lan et al. [12] conducted *in vitro* and *in vivo* experiments to investigate the impact of breviscapine on the progression of hepatic steatosis, inflammation, and fibrosis under metabolic stress. Their results suggest that breviscapine prevents the progression of nonalcoholic steatohepatitis (NASH) by directly inhibiting TAK1 signaling, highlighting its potential as a therapeutic candidate for treating NASH.

Based on these reports, it can be tentatively inferred that the polyphenolic compounds derived from *Erigeron breviscapus* possess a protective effect on liver injury.

Overall, although studies have confirmed that the ethanol and water extracts of *Erigeron breviscapus* have anti fibrosis activity, the specific bioactive components and targets that exert anti fibrosis effects are still unclear. Therefore, elucidating the underlying mechanisms of how these compounds can reverse liver fibrosis is crucial. In the context of the rapid advancements in bio-informatics and pharmacology, network pharmacology has emerged as a highly efficient predictive methodology within the realm of pharmacological research for drug development [13]. This approach emphasizes the examination of intricate relationships among compounds, proteins / genes, and diseases from a holistic network perspective. It has garnered considerable attention within the drug discovery domain owing to its capacity to reveal underlying “protein /gene-disease complex” pathways.

As an emerging discipline, network pharmacology is devoted to the construction of databases and network models. By conducting thorough analyses of these networks, coupled with *in vivo* and *in vitro* experimental validations as well as computer simulations, it aims to elucidate the progression of diseases and the mechanisms of action of drug molecules. A notable characteristic of network pharmacology is its ability to decipher complex network structures that are intertwined with multiple components, pathways, and targets,

thereby demonstrating the attributes of multi-component synergism, multi-pathway engagement, and multi-target modulation [14]. Moreover, network pharmacology utilizes "drug-target-disease" network maps to visually represent the interconnections among components, targets, and pathways, enabling the rapid prediction of potential biological mechanisms. The present study is centered on exploring the therapeutic potential of polyphenolic compounds derived from *Erigeron breviscapus* in the context of human liver fibrosis and associated diseases.

Through a comprehensive literature review and network pharmacological analysis, this paper have predicted the active ingredients, targets, and signaling pathways of these polyphenolic compounds in reversing liver fibrosis and related diseases *in vivo*.

Furthermore, this paper have conducted molecular docking simulations to verify the interactions between the primary active components and core proteins. The objective of this research is to provide theoretical support for the application of polyphenolic compounds from *Erigeron breviscapus* in the treatment of liver fibrosis and ultimately contribute to the development of novel therapeutic strategies aimed at alleviating liver fibrosis and facilitating liver damage repair.

2 Materials and methods

2.1 Materials and reagents

In the preliminary stage, this paper collected the effective active ingredients of *Erigeron breviscapus* by consulting relevant literature. Subsequently, using the TCMSP (Version 2.3) platform, not only were the chemical names of these active ingredients retrieved, but their corresponding CAS numbers were also successfully obtained. During this process, the screening criteria were set as oral bio-availability (OB) reaching or exceeding 30%, and drug likeness index (DL) not less than 0.18. Subsequently, research shifted towards SwissTarget Prediction (http: // www. Swisstarget prediction. ch/) (Version 2013). The database is used for target prediction, and only targets with non-zero probabilities are selected as research objects. In addition, attempts were made to obtain the 2D structure of the active ingredient from the PubChem (Version 2024) database and use it in swiss ADME (http://www.swiss adme.ch) (Version 2024). On the platform, further screening is conducted based on the criteria of GI absorption being "high" and at least two indicators in Drug likeness being "yes", and then SwissTarget Prediction is used again (http://www.swiss adme.ch/) (Version 2024). Perform target prediction. If the above methods fail to obtain sufficient data, additional steps should be taken: using PubChem to retrieve the corresponding Canonical SMILES number based on the CAS number, and continuing to search for targets with non-zero likelihood in SwissTarget Prediction. For active substances that cannot be predicted by Swiss Target Prediction, the authors turned to the TCMSP platform to obtain their target information and utilized Uniprot (https: //www. uniprot. org/uploadlists) (Version 2024). The database confirms the gene names corresponding to these targets. Finally, all collected targets were sorted and deduplicated to determine the key functional targets of the polyphenolic active ingredients in *Erigeron breviscapus*.

2.2 Screening of liver fibrosis targets

The author used GeneCards disease database (https://www.genecards.org/, Version 5.24) Search with the keyword "Liver fibrosis" and screen with a "correlation score" of 25 to obtain disease targets.

2.3 Acquisition of intersection targets and construction of PPI network

The researcher will incorporate the frequently analyzed targets into the String database (Version 12). Simultaneously, a target network diagram is formulated, specifying "Homo sapiens" as the focal species. Subsequently, the formulated PPI (protein-protein interaction) network is introduced into Cytoscape version 3.10.1 software for the purpose of identifying the core targets associated with

Erigeron breviscapus. Within Cytoscape 3.10.1, the CytoNCA plugin is utilized, opting for the "without weight" option to compute the degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) of the targets. This process allows for the selection of key targets, ultimately leading to the identification of the core protein.

2.4 Analysis of GO and KEGG pathway Enrichment

The intersecting targets were imported into the DAVID platform (https: //david.ncifcrf. gov/summary. jsp, Version 6.8). The select identifier and select species are set to "OFFICIAL_ENE_SYMBOL" and "Homo sapien srespectively". Perform GO and KEGG enrichment analysis. As a result, GO bar charts and KEGG bubble charts were created using the Wei sheng Xin website. Using Cytoscape_v3.10.1 software, construct a network diagram of "*Erigeron breviscapus* main active ingredients targets pathways liver fibrosis".

2.5 Molecular docking

The three-dimensional structures of each active ingredient, in SDF format, were retrieved from the Pub Chem database and subsequently converted to .mol2 files. The corresponding pdb format of the target protein was acquired from the PDB database (Version 2024). Pymol (Version 2.5.7) software was employed to eliminate water molecules and ligands, and to facilitate visualization. For the purpose of hydrogenation, molecular docking, and binding energy calculation between the active ingredient and protein, AutoDockTools (version 1.5.7) was utilized, with the "Genetic Algorithm" chosen as the computational method.

3 Results and discussion

3.1 Polyphenolic active ingredients and targets of Erigeron breviscapus

Through a systematic literature search and organization process, a comprehensive list of 49 polyphenolic active ingredients was compiled from *Erigeron breviscapus*. According to strict screening criteria, 12 eligible components were summarized and listed, labeled as DZXX1 to DZXX12, as shown in Table 1. Furthermore, a total of 184 target genes were successfully identified.

Table 1 The main active components of *Erigeron breviscapus*

Mol ID	Molecule name	OB/%	DL	Degree
MOL000006	luteolin	36.16	0.25	15
MOL000098	quercetin	46.43	0.28	11
MOL000392	formononetin	69.67	0.21	8
MOL000422	kaempferol	41.88	0.24	14
MOL000816	ergosta-7,22-dien-3-one	44.88	0.72	5
MOL001040	(2*R*)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21	12
MOL002712	6-Hydroxykaempferol	62.13	0.27	14
MOL002714	baicalein	33.52	0.21	12
MOL002914	Eriodyctiol (flavanone)	41.35	0.24	12
MOL005922	Acanthoside B	43.35	0.77	11
MOL007963	1-hydroxy-2,3,5-trimethoxy-xanthone	101.06	0.30	8
MOL007984	Δ5,22-stigmastadien-3-ol	43.83	0.76	2

3.2 Common target screening

From the Genecards, OMIM, and TTD databases, this paper gathered a total of 8375 relevant targets pertaining to liver fibrosis. To investigate the potential inhibitory effects of *Erigeron breviscapus* polyphenolic components on liver fibrosis, this paper employed a Venn diagram created on the Wei sheng Xin platform to visualize the intersection between the component targets and liver fibrosis targets. This approach yielded 67 common targets, as depicted in Fig. 1.

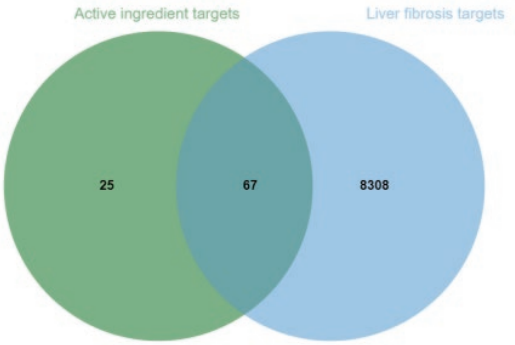


Fig. 1 Venn diagram of *Erigeron breviscapus* Polyphenols and liver fibrosis targets

3.3 Analysis of the network diagram of polyphenols, active ingredients, and target points in Erigeron breviscapus

A network diagram of "polyphenol active ingredient targets" for 67 common targets was constructed using Cytoscape-v3.10.1, including 117 nodes and 153 edges. Calculate the degree value of each node through CytoNCA analysis. The higher the degree value, the greater the possibility of the compound exerting therapeutic effects, as shown in Fig. 2. Among them, the red triangle represents Scutellaria baicalensis, the red circle represents the different active ingredients of Scutellaria baicalensis, and the light yellow diamond represents the target protein. Each edge represents the interaction relationship between the active ingredient and the target, as well as between the active ingredient and the target. Among them, the larger the degree value, the darker the colo.

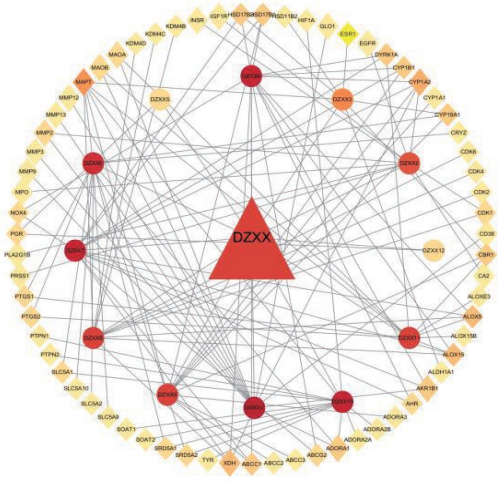


Fig. 2 Network diagram of polyphenols active ingredients target in *Erigeron breviscapus*

3.4 Target protein interaction network of polyphenols from Erigeron breviscapus reversing liver fibrosis

After screening, 11 core protein targets were obtained. The screening process is shown in Fig. 3. With the screening order being BC, CC, and DC. Faint yellow represents not meeting the screening criteria and will not participate in the next step of screening, while yellow represents meeting the screening criteria and can proceed to the next tep of screening. In addition, the larger the Degree value, the more circles are represented in the Figure. The larger the radius. After screening, EGFR, ESR1, PTGS2, HIF1A, and MMP9 showed the strongest biological correlation.

The protein-protein interaction analysis of the intersecting targets is shown in Fig. 4. Each node represents a protein, and each edge represents its different interactions. Involving a total of 67 nodes and 287 edges. The large number of interactions between targets suggests that they may have biological correlations, and the inhibition of liver fibrosis and disease by the active ingredients of *Erigeron breviscapus* polyphenols may involve multiple targets. After analysis and processing with Cytoscape, the selection conditions obtained were BC ≥ 43.88, CC ≥ 0.011, DC ≥ 15.22.

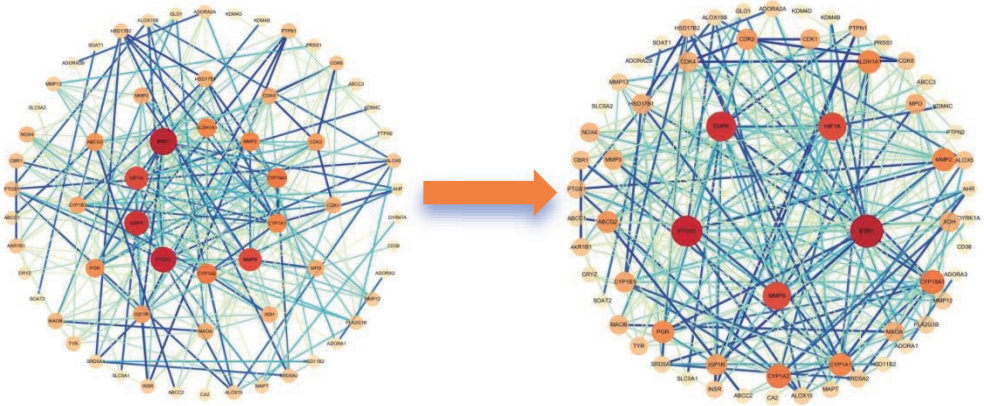


Fig. 3 Screening process of CytoNAC

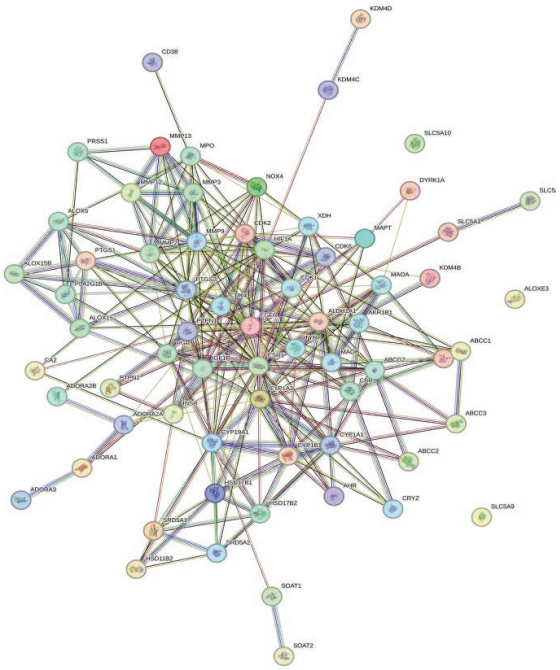


Fig. 4 Network diagram of protein-protein interaction

3.5 Enrichment analysis of GO and KEGG pathways

By examining 67 cross-targets, the author identified a total of 389 entries for GO functional enrichment analysis. Specifically, among these entries, 202 pertained to biological processes (BP), 101 to molecular functions (MF), and 39 to cellular components (CC). The degree of enrichment exhibited a negative correlation with the *P*-values of the entries, prompting their arrangement in ascending order based on *P*-values. Subsequently, the top 10 entries from each category (BP, MF, CC) were chosen for visualization in bar charts, as depicted in Fig. 5.

Regarding biological processes, the polyphenols were predominantly implicated in various functions, including xenobiotic metabolism, responses to external stimuli, lipid metabolism, linoleic acid metabolism, the negative regulation of apoptosis, the positive regulation of miRNA transcription, hepoxilin biosynthesis, the positive regulation of DNA-templated transcription, intracellular receptor signaling pathways, and the positive regulation of cell proliferation, among others.

As for cellular composition, the polyphenols derived from *Erigeron breviscapus* primarily interacted with receptor complexes, endoplasmic reticulum membranes, apical plasma membranes, axons, voltage-gated potassium channel complexes, neuronal cell bodies, extrinsic plasma membrane cytoplasmic components, extracellular spaces, cytosolic fluids, plasma membranes, and plasma membrane caveolae-like invaginations. The molecular functional analysis revealed that the targets of *Erigeron breviscapus* polyphenols were mainly enriched in activities such as nuclear receptor binding, ferric ion binding, delayed rectifier potassium channel activity, steroid binding, ATP binding, enzyme binding, estrogen-responsive element binding, heme binding, transcriptional coactivator binding, carbonic anhydrase activity, and mono oxygenase activity.

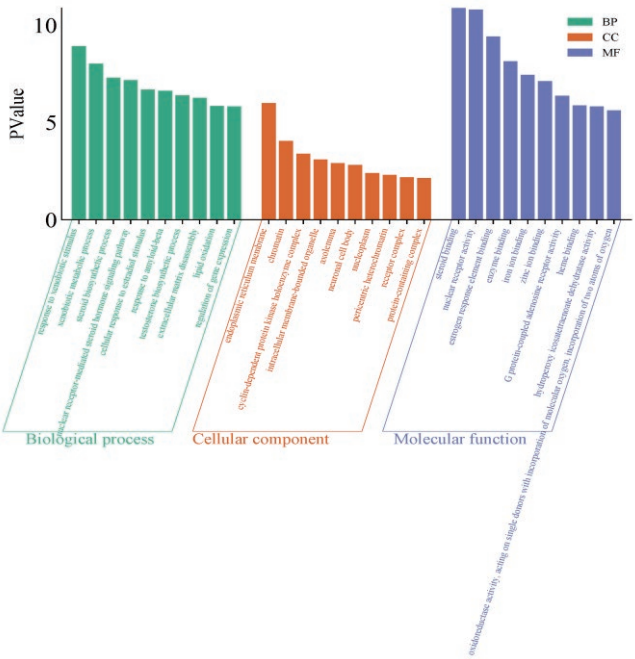


Fig. 5 GO function enrichment analysis Histogram of intersection targets

3.6 KEGG pathway enrichment analysis

KEGG pathway enrichment analysis revealed that 64 intersecting targets were enriched in 49 pathways. The *P* values are sorted in ascending order, and the top 20 signalling pathways are selected to draw a bubble chart. As shown in Fig. 6, the size of bubbles was positively correlated with the number of enriched genes in the signaling pathway. The redder the color of bubbles, the more significant the signaling

pathway. The *X*-axis represents the false discovery rate, which decreases as the *X*-axis moves to the right. The results revealed that the main target of action were enriched in metabolic pathways (26 targets); Pathways in cancer (12 targets); steroid hormone biosynthesis (10 targets); ovarian steroidogenesis; chemical carcinogenesis-reactive oxygen species (9 targets); Arachidonic acid metabolism (8 targetProstate cancer, Endocrine resistance, Serotonergic synapse, Breast cancer (7 targets); Estrogen signaling pathway, Proteoglycans in cancer, Bile secretion, (6 targets); Tryptophan metabolism, Chemical carcinogenesis-DNA adducts (5 targets).

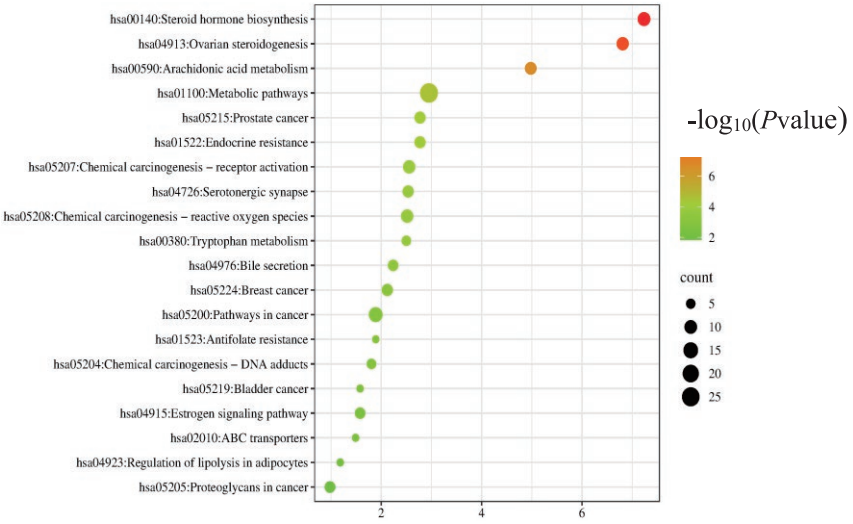


Fig. 6 KEGG function enrichment analysis

3.7 Molecular docking

To demonstrate the effective alleviation of liver fibrosis by polyphenols from *Erigeron breviscapus*, active ingredients and core target proteins were docked on a computer, and their binding efficiency was calculated. Molecular docking was performed between the key active ingredients selected from *Erigeron breviscapus*, including luteolin, 6-Hydroxycaempferol, quercetin, Formononetin, and Kaempferol, and the top five key targets identified through screening in section 3.4: EGFR, ESR1, PTGS2, HIF1A, and MMP9 core targets. The results showed that all core targets could bind to the corresponding compounds. When the molecular docking binding energy is less than 0, it indicates that the two molecules have spontaneous binding ability. When the molecular docking binding energy is less than -1.2 kcal/mol (-5.0 kJ/mol), it indicates good binding between the two molecules [15].

The docking results between the active ingredients and targets of polyphenols in *Erigeron breviscapus*, as visualized in Figs. 7-11, demonstrate the binding efficiency. The selected polyphenolic active ingredients of *Erigeron breviscapus* can bind well with the selected targets, with blue representing the active ingredients and yellow representing hydrogen bonds. The results indicate that the core target binds well to the compound, demonstrating strong ability to bind to the core target (Table 2). For example, luteolin binds to MMP9 with a binding energy of -4.11 kcal/mol; Kaempferol binds to HIF1A with a binding energy of -4.02 kcal/mol; The binding energy between Formononetin and MMP9 is -6.57 kcal/mol; 6-Hydroxykaempferol binds to MMP9 with a binding energy of -4.34 kcal/mol; Quercetin binds to MMP9 with a binding energy of -4.56 kcal/mol, etc. The final docking visualization effect is shown in Fig. 12.

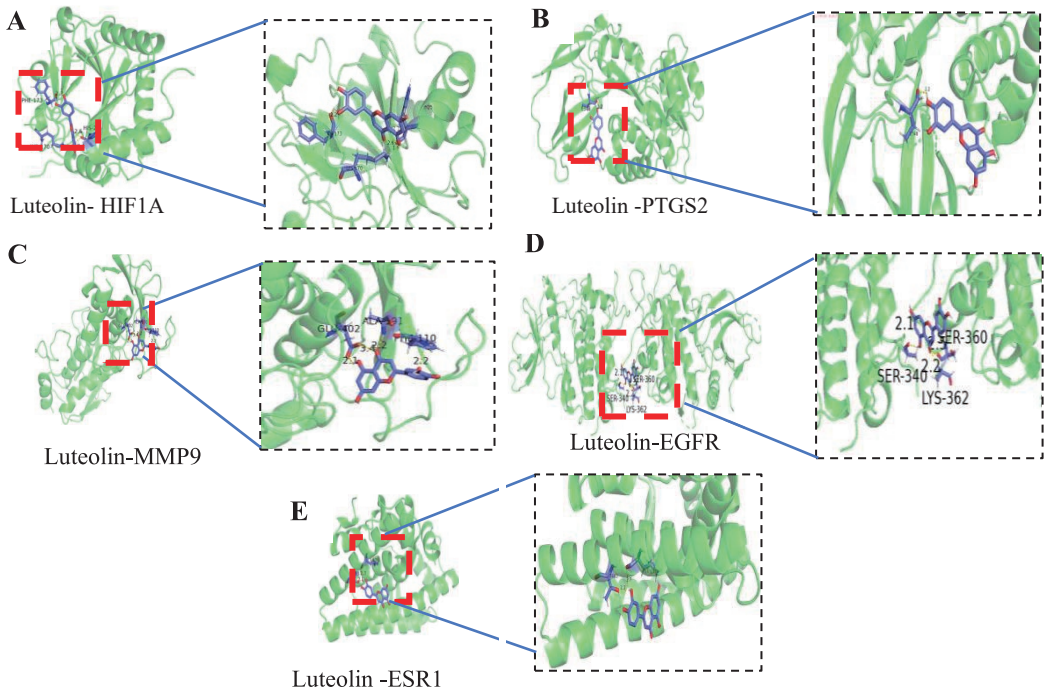


Fig. 7 Molecular docking results. A-E are the docking results of Luteolin with HIF1A, PTGS2, MMP9, ESR1, and EGFR, respectively A: The binding mode of HIF1A and luteolin; B: The binding mode between PTGS2 and luteolin; C: The binding mode between MMP9 and luteolin; D: The binding mode of EGFR and luteolin; E: The binding mode of ESR1 and luteolin.

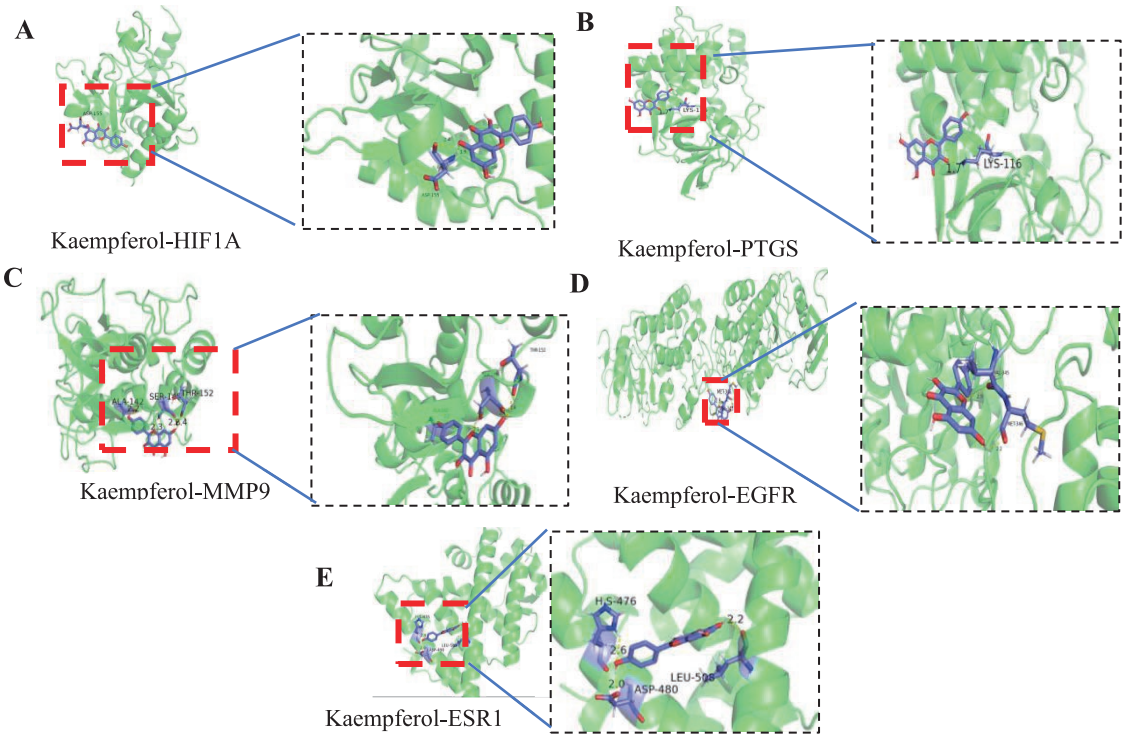


Fig. 8 Molecular docking results. A-E are the docking results of Kaempferol with HIF1A, PTGS2, MMP9, ESR1, and EGFR, respectively A: The binding mode of HIF1A and Kaempferol; B: The binding mode between PTGS2 and Kaempferol; C: The binding mode between MMP9 and Kaempferol; D: The binding mode of EGFR and Kaempferol; E: The binding mode between ESR1 and Kaempferol.

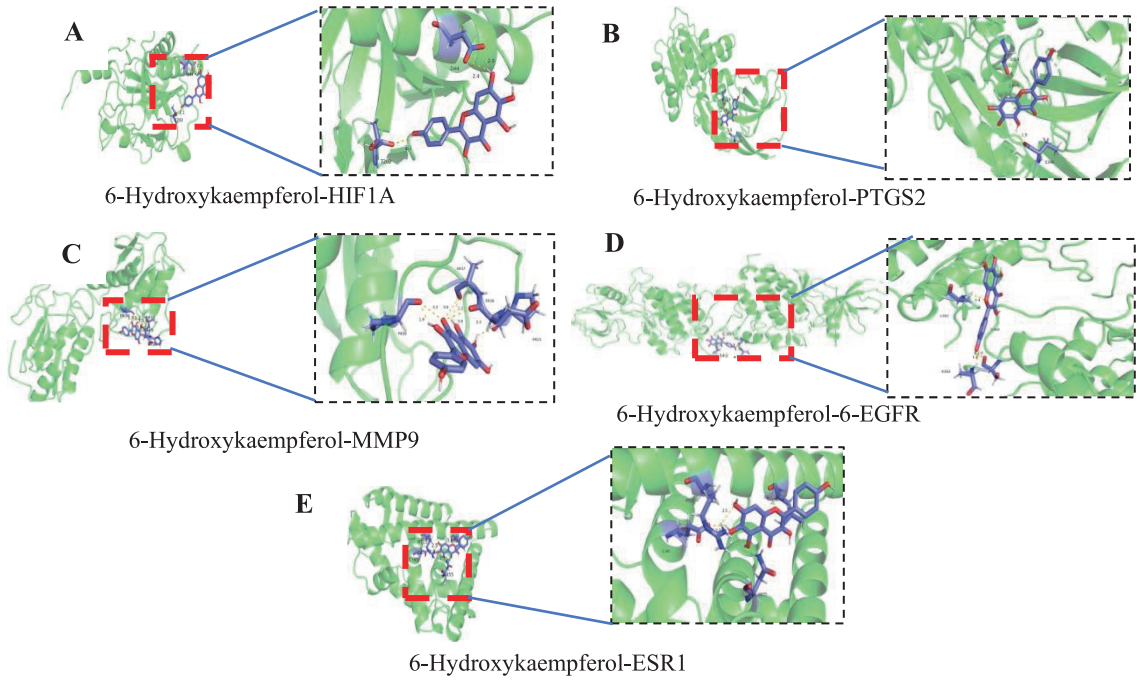


Fig. 9 Molecular docking results. A-E are the docking results of 6-Hydroxykaempferol with HIF1A, PTGS2, MMP9, ESR1, and EGFR, respectively A: The binding mode of HIF1A and 6-Hydroxykaempferol; B: The binding mode between PTGS2 and 6-Hydroxykaempferol; C: The binding mode between MMP9 and 6-Hydroxykaempferol; D: The binding mode of EGFR and 6-Hydroxykaempferol ; E: The binding mode of ESR1 and 6-Hydroxykaempferol.

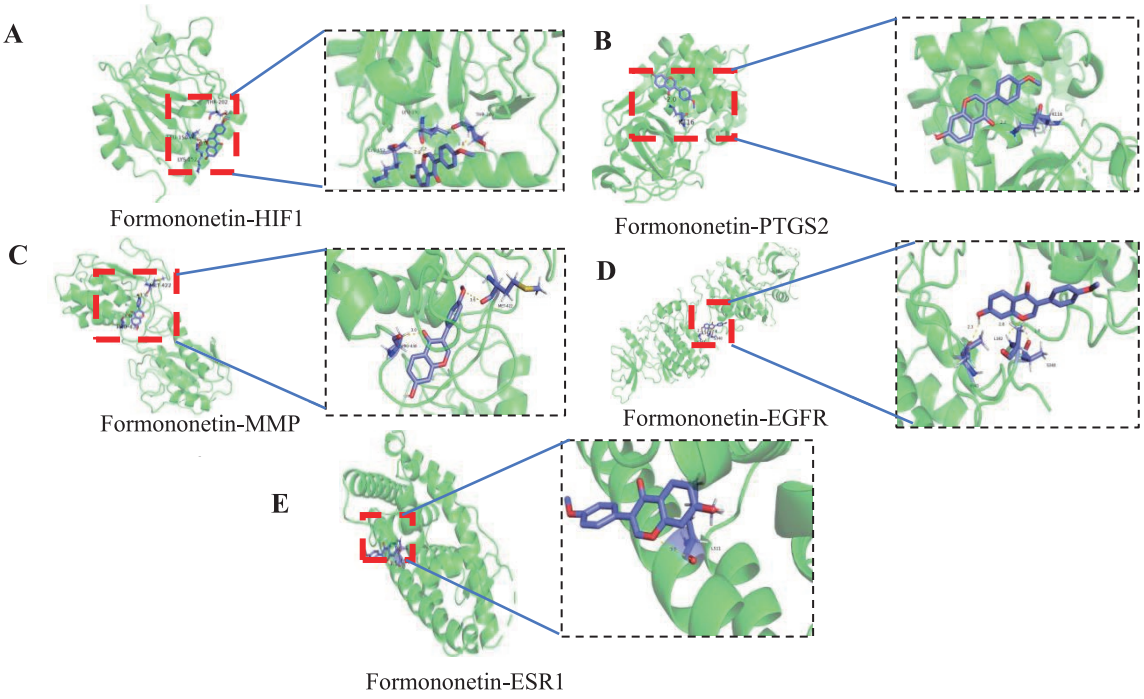


Fig. 10 Molecular docking results. A-E are the docking results of Formononetin with HIF1A, PTGS2, MMP9, ESR1, and EGFR, respectively A: The binding mode of HIF1A and Formononetin; B: The binding mode between PTGS2 and Formononetin; C: The binding mode between MMP9 and Formononetin; D: The binding mode of EGFR and Formononetin ; E: The binding mode of ESR1 and Formononetin.

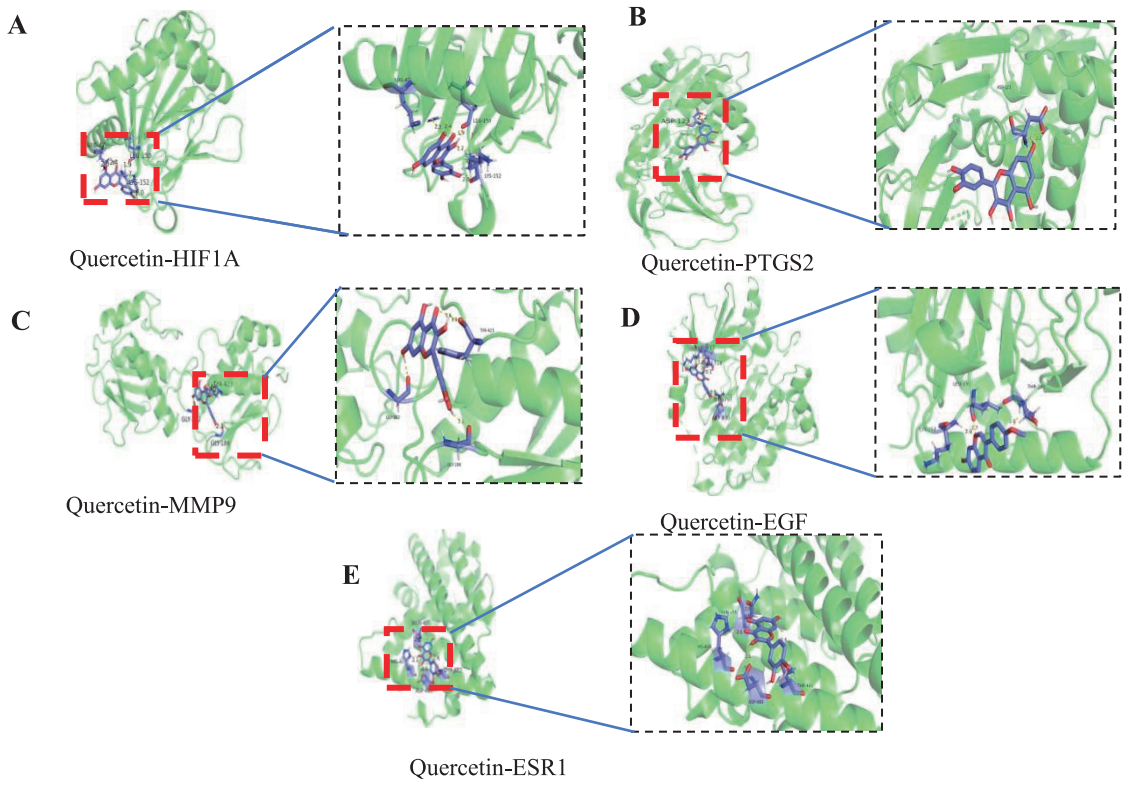


Fig. 11 Molecular docking results. A-E are the docking results of Quercetin with HIF1A, PTGS2, MMP9, ESR1, and EGFR, respectively. A: The binding mode of HIF1A and Quercetin; B: The binding mode between PTGS2 and Quercetin; C: The binding mode between MMP9 and Quercetin; D: The binding mode of EGFR and Quercetin ; E: The binding mode of ESR1 and Quercetin.

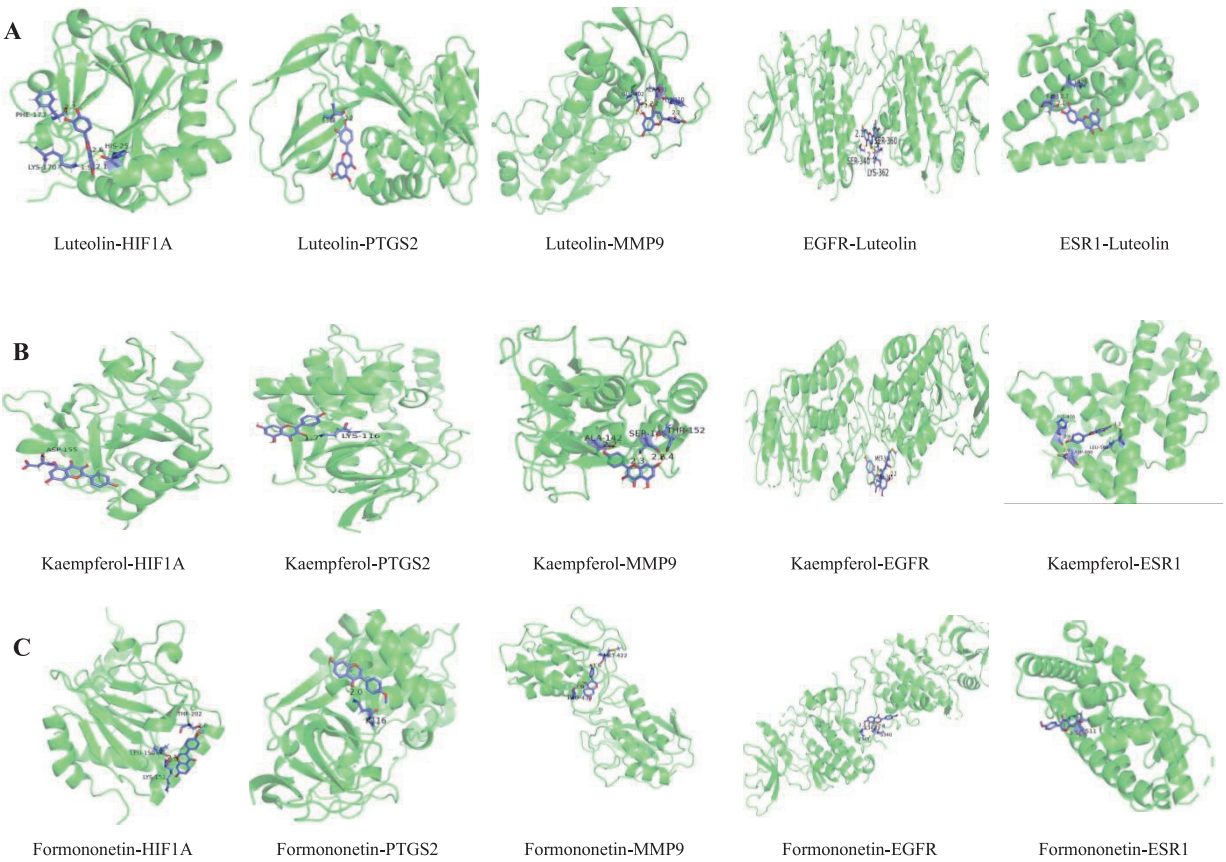
Table 2 The binding energy between the main active ingredients of *Erigeron breviscapus* and the core target molecule docking

Active ingredient name	Protein target name	Binding energies / (kcal/mol)
Luteolin	ESR1 (PDB ID: 5gS4)	-3.38
	EGFR (PDB ID: 2rf9)	-3.81
	MMP9 (PDB ID: 1gKc)	-4.11
	PTGS2 (PDB ID: 2VNA)	-2.81
	HIF1A (PDB ID: 3HQR)	-3.85
Kaempferol	ESR1 (PDB ID: 5gS4)	-3.89
	EGFR (PDB ID: 2rf9)	-4.05
	MMP9 (PDB ID: 1gKc)	-3.80
	PTGS2 (PDB ID: 2VNA)	-2.19
	HIF1A (PDB ID: 3HQR)	-4.02
6-Hydroxykaempferol	ESR1 (PDB ID: 5gS4)	-3.81
	EGFR (PDB ID: 2rf9)	-3.58

(to be continued)

Continued Table 2

Active ingredient name	Protein target name	Binding energies / (kcal/mol)
formononetin	MMP9 (PDB ID: 1gKc)	-4.34
	PTGS2 (PDB ID: 2VNA)	-2.34
	HIF1A (PDB ID: 3HQR)	-3.14
	ESR1 (PDB ID: 5gS4)	-4.80
	EGFR (PDB ID: 2rf9)	-4.55
	MMP9 (PDB ID: 1gKc)	-6.57
	PTGS2 (PDB ID: 2VNA)	-3.33
	HIF1A (PDB ID: 3HQR)	-4.64
	ESR1 (PDB ID: 5gS4)	-3.45
	EGFR (PDB ID: 2rf9)	-3.56
Quercetin	MMP9 (PDB ID: 1gKc)	-4.56
	PTGS2 (PDB ID: 2VNA)	-2.46
	HIF1A (PDB ID: 3HQR)	-4.02



Luteolin-HIF1A Luteolin-PTGS2 Luteolin-MMP9 EGFR-Luteolin ESR1-Luteolin

Kaempferol-HIF1A Kaempferol-PTGS2 Kaempferol-MMP9 Kaempferol-EGFR Kaempferol-ESR1

Formononetin-HIF1A Formononetin-PTGS2 Formononetin-MMP9 Formononetin-EGFR Formononetin-ESR1

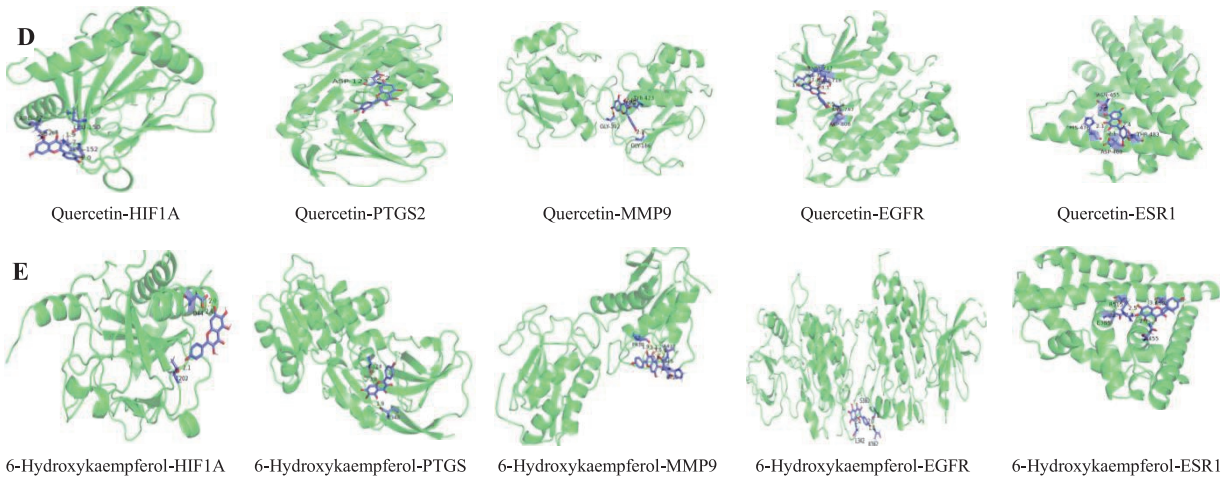


Fig. 12 Molecular docking and visualization of docking results: A: represents the docking and visualization of Luteolin with HIF1A, PTGS2, MMP9, EGFR, and ESR1, respectively; B: represents the docking and visualization of Kaempferol with HIF1A, PTGS2, MMP9, EGFR and ESR1, respectively; C: represents the docking and visualization of Formononetin with HIF1A, PTGS2, MMP9, EGFR, and ESR1, respectively; D: represents the docking and visualization of Quercetin with HIF1A, PTGS2, MMP9, EGFR, and ESR1, respectively; E: represents the docking and visualization of 6-Hydroxykaempfero with HIF1A, PTGS2, MMP9, EGFR, and ESR1, respectively.

4 Discussion

Research has shown that *Erigeron breviscapus* exhibits diverse pharmacological effects, including anti-inflammatory, antioxidant properties, choleretic effects, and blood pressure regulation. However, there is still a lack of in-depth research on its active ingredients and mechanisms in anti liver fibrosis. This study obtained 12 active ingredients of *Erigeron breviscapus* from the TCMSP database, including luteolin, quercetin, formononetin, and kaempferol, among which polyphenolic compounds occupy an important position. Through network pharmacology methods, we screened 49 active ingredients and 153 potential targets, further focusing on 12 core ingredients and 117 core targets for anti-liver fibrosis. These targets involve 379 biological functions and 44 signaling pathways, highlighting the multi-component and multi-target characteristics of the anti-liver fibrosis effect of *Erigeron breviscapus*.

This paper analyzed the "active ingredient potential target" network of *Erigeron breviscapus* for its anti-liver fibrosis properties and found that 12 components, including luteolin, quercetin, and kaempferol, had high degree values and made significant contributions to the network. Therefore, this paper speculate that they may be the main active ingredients of *Erigeron breviscapus* for anti-liver fibrosis. The degree value is greater than 10, indicating significant contributions to the entire network. Based on this, it is speculated that these 8 components are the main functional substances of *Erigeron breviscapus* in combating liver fibrosis.

Quercetin (QCT), also known as 3,3',4',5,7-pentahydroxyflavone, is the most common type of flavonol compound and belongs to the category of natural plant polyphenols [16]. QCT exhibits diverse biological effects and has broad potential applications in the field of pharmacology. It can effectively eliminate active free radicals, bind and capture them, thereby inhibiting the occurrence of lipid peroxidation in the body. In addition, QCT exhibits significant pharmacological activities in antioxidant, anti-inflammatory, antibacterial, antiviral, and prevention of cardiovascular disease complications. Specifically, in a rat model of liver fibrosis induced by carbon tetrachloride, QCT exhibited inhibitory effects on the progression of liver fibrosis and protected the liver [17]. The mechanism is to up regulate the expression levels of interleukin-10 (IL-10) and heme oxygenase-1 (HO-1), effectively inhibiting the activation of NOD like receptor protein 3 (NLRP3) inflammasome and the

release of inflammatory factors, further achieving a protective effect against acute alcoholic liver injury, and effectively alleviating further pathological changes in liver fibrosis.

Luteolin (Lut), is classified as a flavonoid compound and is a dietary flavonoid component that exhibits diverse biological activity characteristics, including multiple pharmacological effects such as anti-inflammatory, anticancer, anti-estrogenic, and induction of cell apoptosis [18]. Osmanthus extract inhibits astrocyte function *in vitro* by suppressing the AKT/mTOR/p70S6K and TGF *β*/Smad signaling pathways, and has anti fibrosis effects.

Formononetin extract, also known as thorn flower extract, is a flavonoid plant estrogen with the chemical name 7-hydroxy-4'-methoxyflavone. It can regulate the internal environment and homeostasis of the human body, improve the endocrine microenvironment, regulate estrogen and metabolism, anti-inflammatory, anti-oxidant, anti-apoptotic, lipid-lowering, anti-thrombotic, etc [19]. In recent years, with the deepening of research, a large number of experimental results have suggested that Formononetin has a certain inhibitory effect on NF-*κ*B activation. Wu et al. found that Formononetin significantly inhibits the proliferation and metastasis of liver cancer cells by inhibiting the TLR 4/NF-*κ*B pathway [20]. Studies have shown that by inhibiting cell growth *in vitro* and *in vivo*, Formononetin successfully inhibits tumor growth *in vivo* by targeting the TNF-*α*/NF-*κ*B pathway in colorectal tumor bearing nude mice [xxxx].

Kaempferol 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one has found that the pharmacological effects of kaempferol include anti-cancer [22], anti-inflammatory [23], antioxidant [23], heart protection [25], neuroprotection [26] and improvement of diabetes [27]. Epidemiological studies have shown that kaempferol can reduce the incidence rate of different types of cancers, including liver, colon, ovary, pancreas, stomach, bladder and other organs, and inhibit cancer progression through a variety of different mechanisms [28]. Saw and others use kaempferol to treat HCC. The results show that kaempferol increases the mRNA and protein expression of antioxidant regulation genes by activating Nrf2-ARE signaling pathway, thereby inhibiting oxidative stress [29]. In another study on HCC, it was found that kaempferol can promote the binding of Nrf2 to ARE sequences, activate the expression of endogenous antioxidants, detoxifying enzymes, and transporters [30]. Therefore, Nrf2 is a potential target for the treatment of various cancers, including HCC, with kaempferol [31].

Breviscapine, it is a natural mixture of flavonoid glycosides isolated from the traditional Chinese medicinal herb *Erigeron breviscapus* [32]. The main active ingredient is baicalin (*Erigeron breviscapus*), which has various pharmacological activities such as antioxidant, anti-inflammatory, anti-fibrotic, and lipid-lowering [33], Breviscapine extract is extracted from the traditional Chinese medicine *Erigeron breviscapus* effective ingredients, with the functions of dispelling wind and dampness, promoting blood circulation and removing blood stasis, activating meridians and collaterals, and antioxidation. Its function is to dilate blood vessels and improve microcirculation in the brain, heart, and liver [34]. Studies have shown that breviscapine can alleviate ventricular remodeling in rats with acute myocardial infarction by regulating the trans forming growth factor-*β*1 (TGF-*β*1) / Smad pathway, suggesting that TGF-*β*1 may be a potential target for Breviscapine to exert its pharmacological effects [35]. TGF-*β*1 is currently recognized as one of the fibrogenic factors in academia, which can synergistically regulate ECM deposition by regulating Smad dependent and non dependent extracellular signal regulated protein kinase (ERK) pathways. Therefore, inhibiting TGF-*β*1 and its related pathways can delay or even reverse the fibrosis process [36]. Smad2 plays an important role in the TGF-*β*1/Smad pathway, inhibiting the activation of Smad2 in liver cells, effectively reducing the expression of TGF-*β*1, and delaying the occurrence and development of HF; at the same time, ERK1 can promote fibrosis progression by phosphorylating Smad2, indicating a signaling interaction between the TGF-*β*1/Smad2 pathway and ERK1. By analyzing the protein interactions of potential targets for the anti-liver fibrosis effect of *Erigeron breviscapus*, it was found

that the top 5 targets in terms of degree were ESR1, PTGS2, EGFR, HIF1A, MMP9.

The full name of ESR1 is estrogen receptor 1, which consists of 8 exons and 7 in trons spanning 140 kb of chromosome 6q25.1 and encodes estrogen receptor α (ERα). ESR1 is the target gene of miR-181a-5p. Inhibiting miR-181a-5p can increase ESR1 expression, thereby increasing ERBB2 expression and reducing liver cell apoptosis and inflammatory damage [37]. MMP9 is a matrix metalloproteinase-9 that plays a crucial role in KC mediated ECM degradation to promote the resolution of liver fibrosis [38]. EGFR is an epidermal growth factor receptor that can activate HSC, inhibit its signaling, and delay or reverse liver fibrosis. PTGS2 plays an important role in regulating immune responses, anti-inflammatory effects, and other aspects. HIFIA can directly up regulate the expression of LOXL1 and promote the activation of fibroblasts during liver fibrosis [39]. It can be seen that the above key targets are all related to liver fibrosis.

The GO functional enrichment results indicate that *Erigeron breviscapus* mainly regulates the metabolic processes of foreign substances closely related to the pathogenesis of liver fibrosis, stimuli response to the outer edge, lipid metabolism, linoleic acid metabolism, negative regulation of apoptosis, positive regulation of miRNA transcription, and other processes. The KEGG enrichment results indicate that *Erigeron breviscapus* plays a role in the treatment of liver fibrosis through various pathways such as metabolic pathways, chemical on cogenic receptor activation pathways, and the pathogenesis of cancer. It is worth noting that a single component may regulate multiple pathways to exert its effects, such as quercetin in the core component, which may act on metabolic pathways, Proteoglycans in cancer signaling pathways, Rap1 signaling pathways, etc. It has been reported that PI3K / Akt signaling pathway [40], Rap1 signaling pathway [41], MAPK signaling pathway [42], Ras signaling pathway [43] are closely related to the treatment of liver fibrosis, which further verifies the accuracy of network prediction. The molecular docking results showed that the selected core components had good docking with the target and could bind freely. Analyze from the perspective of composition, luteolin, formononetin, 6-hydroxycaempferol, quercetin, kaempferol low binding energy with most; from the perspective of target analysis, ESR1 and MMP9 have low binding energies with most components. This result suggests that *Erigeron breviscapus* may exert anti liver fibrosis effects by acting on targets such as EFGR, ESR1, HIF1A etc through compounds such as luteolin, formononetin, 6-hydroxycaempferol, quercetin, kaempferol, etc.

5 Conclusion

In the present study, the mechanism of the synergistic antihepatic fibrosis effects of the multicomponent, target and pathway of *Erigeron breviscapus* was preliminarily elucidated using network pharmacology and molecular docking techniques. Based on the data suggestive of the results of this experiment it is known that *Erigeron breviscapus* may act through: luteolin, 6-hydroxykaempferol, formononetin, quercetin and kaempferol. These active ingredients act on targets such as EFGR, MMP9, ESR1, involved in the regulation of the Rap1 signaling pathway, lipids and atherosclerosis, the MAPK signaling pathway and other pathways. This regulation produces anti-inflammatory effects, inhibits the activation of HSC and promotes their apoptosis, thus exerting anti-fibrosis effects. Therefore, this study provides a reference for the development of new drugs for the treatment of hepatic fibrosis with *Erigeron breviscapus*. In addition, since this study relies heavily on database analysis and has some limitations, it will be further validated by *in vitro* and *in vivo* experiments in subsequent studies.

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