Regular article

Study on the mechanism of *Curcuma zedoaria* in breast cancer based on network pharmacology

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Abstract

This study explored the anti-breast cancer (BC) mechanism of *Curcuma zedoaria* (CZ) via the network pharmacology approach. The active components of CZ were screened by oral bioavailability (OB) and drug-likeness (DL) criteria using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). The potential targets of CZ were predicted through TCMSP and SwissTargetPrediction, while BC-related target genes were screened via GeneCards and the Online Mendelian Inheritance in Man (OMIM) database. The common target genes of CZ and BC were obtained by using the Venn tool. The protein interaction network diagram was constructed by using String software. The network diagram of CZ components was created by using Cytoscape 3.8.0. The intersection genes of drugs and diseases were analyzed by using the R language software for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, the docking activity between the active ingredient and the key target was verified through molecular docking. Through the methods of database search and literature mining, 23 bio-active components of CZ and 370 corresponding gene targets, 1776 disease gene targets, and 132 drug-disease intersection genes were obtained. GO analysis revealed 67 types of cellular components, 113 molecular functions, and 2042 biological processes. KEGG analysis identified 138 enriched pathways. This study preliminarily elucidated the potential mechanism underlying the therapeutic effect of CZ on BC.

Keywords: *Curcuma zedoaria* (CZ); breast cancer (BC); network pharmacology; mechanism of action

1 Introduction

Breast cancer (BC) is currently the most prevalent malignant tumor among women worldwide [1-2] and also one of the most fatal malignancies. According to the latest research, BC is the second leading cause of cancer-related deaths among women [3]. It has a high prevalence and mortality rate, posing a serious threat to the health and lives of women. Clinically standard treatment methods, such as radiotherapy, chemotherapy, and surgical therapy, have certain limitations in the treatment of breast cancer patients, which can easily lead to treatment failure and recurrence. Western BC treatment strategies are mainly based on guidelines from the European Society for Medical Oncology (ESMO, https://www.esmo.org/) and the National Comprehensive Cancer Network (NCCN, https://

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www.nccn.org/), yet inconsistencies exist between these two guidelines in aspects such as genetic risk assessment and treatment recommendations [4]. The current clinical treatment is prone to failure and recurrence, which, to some extent, aggravates the physical and psychological pain of patients and may even affect subsequent treatment. Traditional Chinese Medicine (TCM) is an integral part of Complementary and Alternative Medicine (CAM). Its mild efficacy can alleviate the side effects caused by Western medicine (such as nausea and vomiting) during the treatment process, and it is becoming increasingly popular among breast cancer patients of Chinese descent [5]. Some data has indicated that CZ, combined with the inferior vena cava, can be supplemented during clinical chemotherapy to reduce adverse reactions and prolong the survival period of triple-negative breast cancer [6]. CZ can also be combined with other traditional Chinese medicines for treatment, which is a novel clinical treatment approach [7]. At present, the anti-breast cancer effect of CZ is in the initial exploration stage, and many key genes and pathways have not been experimentally verified. Therefore, more scientific research and clinical trials are needed to lay the foundation for future pharmacological research on CZ's anti-breast cancer effect and better solve the problem of breast cancer treatment. Therefore, utilizing network pharmacology methods to investigate the potential mechanisms of the traditional Chinese medicine CZ in treating breast cancer (BC) holds a specific necessity, providing references for the subsequent development of anticancer drugs.

CZ is used clinically as its dried rhizome, and is mainly distributed in Sichuan, Guangxi, and Yunnan provinces of China. Volatile oils and curcuminoids are the major active components of CZ [8]. The extract of traditional Chinese medicine CZ, known as zedoary oil, contains multiple components, such as curcumenol, β-elemene, and curzerenone, exhibiting multi-target antitumor effects against various malignant tumors, including breast cancer and liver cancer [9-10]. Network pharmacology is a biology-based theoretical approach that performs systematic network analysis of biological systems, characterized by a "multi-component, multi-target" drug-disease interaction model [11-12]. In this study, we adopted network pharmacology to identify key nodes in the CZ-BC interaction network and elucidate the underlying anti-tumor mechanism, aiming to provide a reference for the exploration of potential therapeutic targets and subsequent experimental verification.

2 Method

2.1 Obtain the bioactive components of CZ

CZ-related chemical components were retrieved from the TCMSP platform (https://www.91tcmsp.com) by inputting the keyword "*Curcuma zedoaria*", and screened with the criteria of human oral bioavailability (OB $\geq$ 30%) and drug-likeness (DL $\geq$ 0.10). In addition, domestic and international literature databases were mined to supplement the anti-BC active components of CZ that have been reported in previous studies. The bioactive components obtained from the above two approaches were collated and integrated to establish a CZ active component database.

2.2 Obtain the relevant targets of CZ

To obtain as many effective targets as possible, screening was conducted by using two methods: (1) The active ingredients screened in Section 2.1 were used to identify corresponding potential protein targets on the TCMSP platform, with the UniProt database (https://www.uniprot.org/) employed

to convert protein gene names into standardized gene names uniformly. (2) The active ingredients screened were used to obtain corresponding 2D structural diagrams through the PubMed database (https://pubmed.ncbi.nlm.nih.gov/). The 2D structural diagrams were imported into the SwissTargetPrediction (http://swisstargetprediction.ch/) to predict potential drug targets with a probability greater than 0. After merging and removing duplicates from the aforementioned identified targets, potential targets were obtained.

2.3 Obtain breast cancer disease-related targets

BC-related target genes were screened from the GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/) databases with the keyword "breast neoplasm". Genes with a relevance score above the 50th percentile were selected for two rounds of screening, and the retrieved BC-related targets were collated with duplicate entries removed to obtain the final BC target gene set.

2.4 Obtain the common target genes of CZ and BC

The targets of drugs and diseases obtained under items 2.2 and 2.3 were screened for intersecting target genes by using the Venn tool, and a Venn diagram was generated.

2.5 Constructing protein-protein interaction network diagrams (PPI)

The overlapping target genes were imported into the STRING database (https://cn.string-db.org/), with the species set to "Homo sapiens" and the minimum interaction confidence threshold set to 0.7 (medium confidence). Other parameters were kept as default. The core targets were screened by the degree value, and a PPI network of CZ against BC was constructed.

2.6 Components-Putative targets network

Cytoscape 3.8.0 software was used to construct a CZ component-potential target network based on the correlation between CZ active components and the overlapping target genes, and to analyze the interaction relationship between components and targets.

2.7 GO analysis and KEGG pathway enrichment analysis

The R language package "clusterProfiler" was used to convert the gene symbols of the overlapping genes into ENTREZ IDs and construct a target gene list. GO functional annotation (including Biological Process [BP], Cellular Component [CC], and Molecular Function [MF]) was performed for the overlapping genes using the functions in the "clusterProfiler" package, and the annotation results were visualized. KEGG pathway enrichment analysis was conducted with the "enrichKEGG" function of the "clusterProfiler" package (https://www.genome.jp/kegg/), and the enriched pathways were visualized using the "pathview" package. According to the adjusted *P* value (the smaller the value, the more significant the enrichment), the top 10 GO terms and top 20 KEGG pathways were selected for visualization.

2.8 Molecular docking

We got the active ingredient's sdf from PubChem (https://pubchem.ncbi.nlm.nih.gov/), then searched the protein's crystal structure on RCSB PDB (https://www.rcsb.org/). We imported both into MOE 2019, minimized the ligand's charge, and cleaned up the protein by removing water and small

molecules. Molecular docking was then performed, and the binding affinity between ligands and receptors was evaluated by the Affinity value.

3 Result

3.1 Screening of active ingredients in CZ

A total of 17 active components of CZ were screened from the TCMSP database according to the criteria of OB ≥ 30% and DL ≥ 0.10. Six additional anti-BC active components of CZ were supplemented by mining domestic and international literature databases (Table 1). All these components have been verified to exert anti-BC effects in modern pharmacological experiments, and a total of 23 bioactive components of CZ were finally identified (Table 2).

Table 1 Domestic and foreign literature databases to supplement

Serial number	Molecule name	Inhibiting breast cancer cells effect	References
1	Beta-elemene	< 40 μmol/L	[13]
2	Curcumin	competitive inhibitory effect	[14]
3	Germacrone	significantly inhibits (0-400 μM)	[15]
4	Curdione	(IC_{50})125.632 μg/mL	[16]
5	Daucosterol	(IC_{50})MCG803 cells: 19.96 μM (IC_{50})BGC823 cells: 3.13 μM (IC_{50})AGS cells: 24.19 μM (IC_{50})MCF-7 cells: 16.95 μM	[17]
6	Demethoxycurcumin	significantly inhibits	[18]

Table 2 CZ active ingredient compounds conforming to OB and DL value ranges

Serial number	Mol ID	Molecule name	OB (%)	DL
1	MOL000901	Curcumenol	87.82	0.13
2	MOL000889	Isocurcumenol	97.67	0.13
3	MOL000898	Curcumenone(1*S*,6*R*,7*R*)-4-isopropylidene-1-methyl-7-(3-oxobutyl)norcaran-3-one	34.17	0.11
4	MOL000474	(-)-Epoxycaryophyllene	35.94	0.13
5	MOL000902	Curcumol	103.55	0.13
6	MOL000938	Calarene	51.55	0.11
7	MOL000915	(1*S*,10*S*),(4*S*,5*S*)-germacrone-1(10),4-diepoxide	30.48	0.18
8	MOL000296	Hederagenin	36.91	0.75

(to be continued)

Continued Table 2

Serial number	Mol ID	Molecule name	OB (%)	DL
9	MOL000940	Bisdemethoxycurcumin	77.38	0.26
10	MOL000208	(+)-Aromadendrene	55.74	0.10
11	MOL000906	Wenjine	47.93	0.27
12	MOL000891	(1*R*,10*R*)-epoxy-1,10-dihydrocurdione	36.73	0.12
13	MOL000897	(3*S*,3a*R*,8a*R*)-3,8a-dihydroxy-5-isopropylidene-3,8-dimethyl-1,2,3a,4-tetrahydroazulen-6-one	38.70	0.12
14	MOL000900	(5*R*,6*R*)-5-isopropenyl-3,6-dimethyl-6-vinyl-5,7-dihydrobenzofuran-4-one	57.05	0.11
15	MOL000935	Hepanal	53.83	0.10
16	MOL000937	58870_FLUKA	49.01	0.10
17	MOL000295	Daucosterol	20.63	0.63
18	MOL000908	Beta-elemene	25.63	0.06
19	MOL000090	Curcumin	5.15	0.41
20	MOL000910	Germacrone	32.50	0.07
21	MOL004255	Curdione	38.94	0.08
22	MOL000903	Gweicurculactone	42.92	0.14
23	MOL001603	Demethoxycurcumin	4.37	0.33

3.2 Collection of curcuma-related targets

Potential protein targets corresponding to the 23 CZ bioactive components were predicted by two methods: TCMSP platform retrieval and SwissTargetPrediction based on 2D structural diagrams (from PubMed). The targets obtained from the two methods were integrated, and duplicate entries were removed, resulting in a total of 370 potential protein targets of CZ.

3.3 Collection of breast cancer targets

A total of 15,606 BC-related targets were retrieved from the GeneCards database, and 1,751 targets with a relevance score > 11 were selected as potential BC targets. In addition, 444 BC-related targets were supplemented from the OMIM database. After merging the two datasets and removing duplicate entries, a total of 1,766 BC-related target genes were finally obtained.

3.4 Collection of intersection genes between CZ and BC

Based on the Venny 2.1 software, the relationships between drug targets and disease targets were constructed, resulting in 132 cross-targets. It was believed that Curcuma longa may exert anti-cancer effects on breast cancer through these intersecting genes. Yellow represents breast cancer, and blue represents CZ. (Fig. 1)

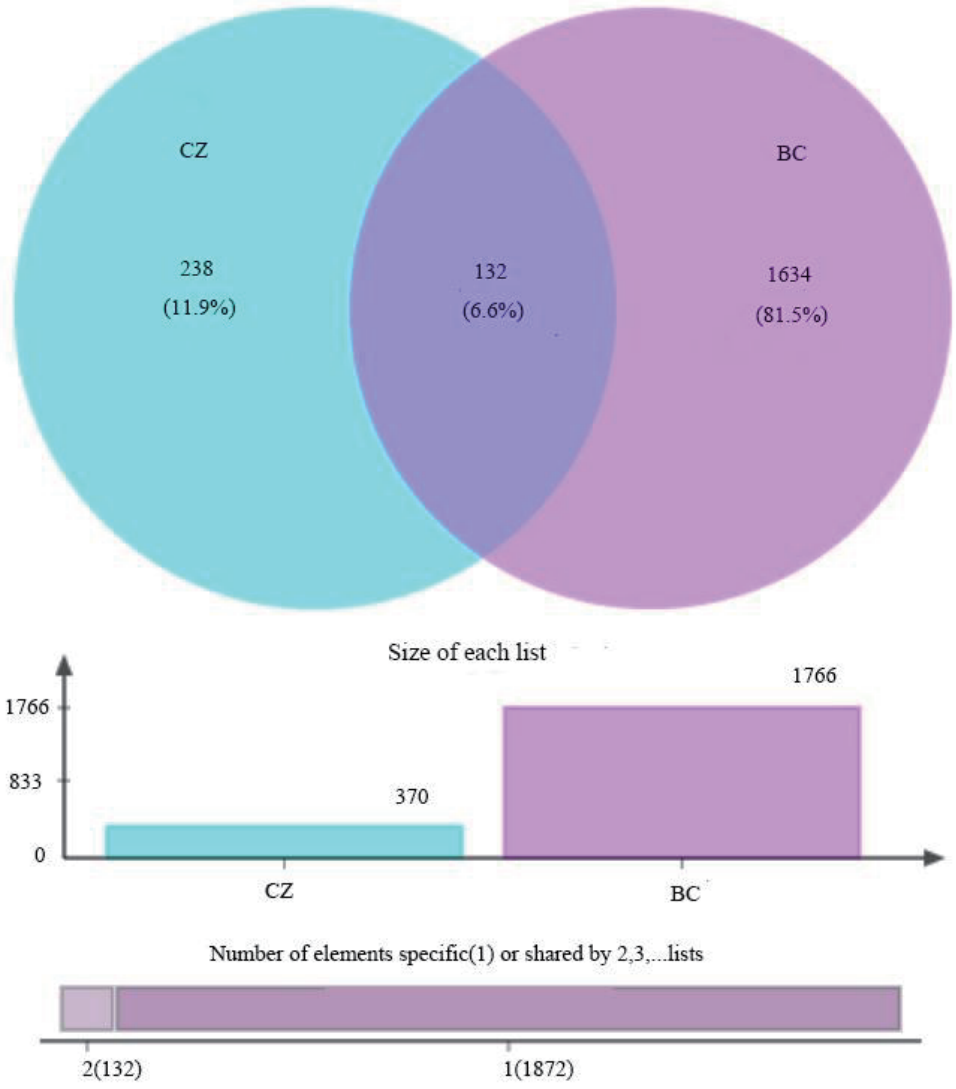


Fig. 1 Venn diagram of intersecting genes between CZ and BC

3.5 CZ composition—BC disease—target network diagram construction

The data files of CZ active components and overlapping genes were constructed and imported into Cytoscape 3.8.0 software to build a CZ component-BC-target network (Fig. 2). In the network diagram, circles represent CZ, squares represent BC, octagons represent CZ active components, and triangles represent overlapping genes. The Network Analyzer plugin was used to analyze the network topology parameters, and the network data table was exported. The network contained a total of 154 nodes and 422 edges with an average degree of 14.5. A higher degree value indicates that the active component interacts with more overlapping genes. According to the degree ranking, the top five key active components of CZ against BC were demethoxycurcumin (degree = 47), bisdemethoxycurcumin (degree = 41), curcumenone (degree = 38), curcumin (degree = 34), and hederagenin (degree = 31). These components

are considered the core active constituents of CZ for BC treatment, which further confirms the "multi-component, multi-target" characteristic of CZ in anti-BC therapy.

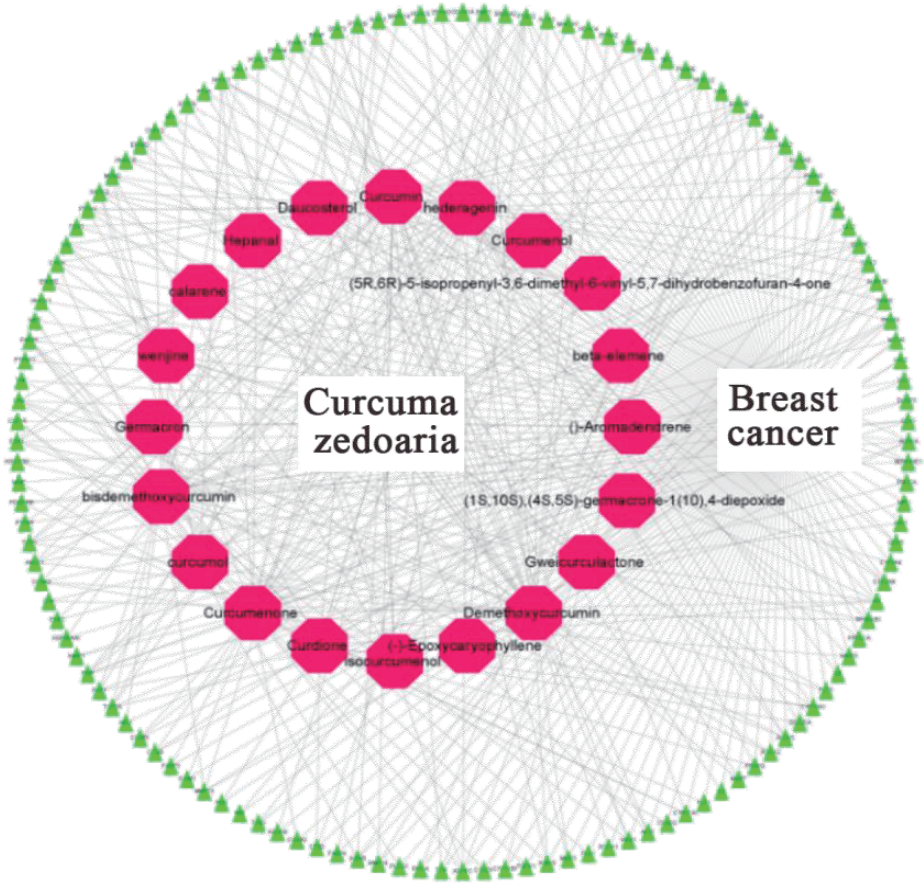


Fig. 2 CZ component—BC disease—target network diagram

3.6 Protein-protein interaction network (PPI) mapping and key gene analysis

The 132 overlapping genes were imported into the STRING database, with the species set to "Homo sapiens" and non-interacting proteins hidden, to construct a PPI network (Fig. 3). Cytoscape 3.8.0 software was used to perform topological analysis of the PPI network, and the top 30 core genes were selected for degree value ranking and bar chart visualization (Fig. 4). In the bar chart, the vertical axis represents the core gene symbols, and the horizontal axis represents the degree value (number of adjacent nodes) of each gene. The top ten core genes with the highest degree values were AKT1, EGFR, CCND1, STAT3, HSP90AA1, IL6, SRC, ESR1, ERBB2, and MTOR. These core genes are considered to play a pivotal role in the anti-BC mechanism of CZ.

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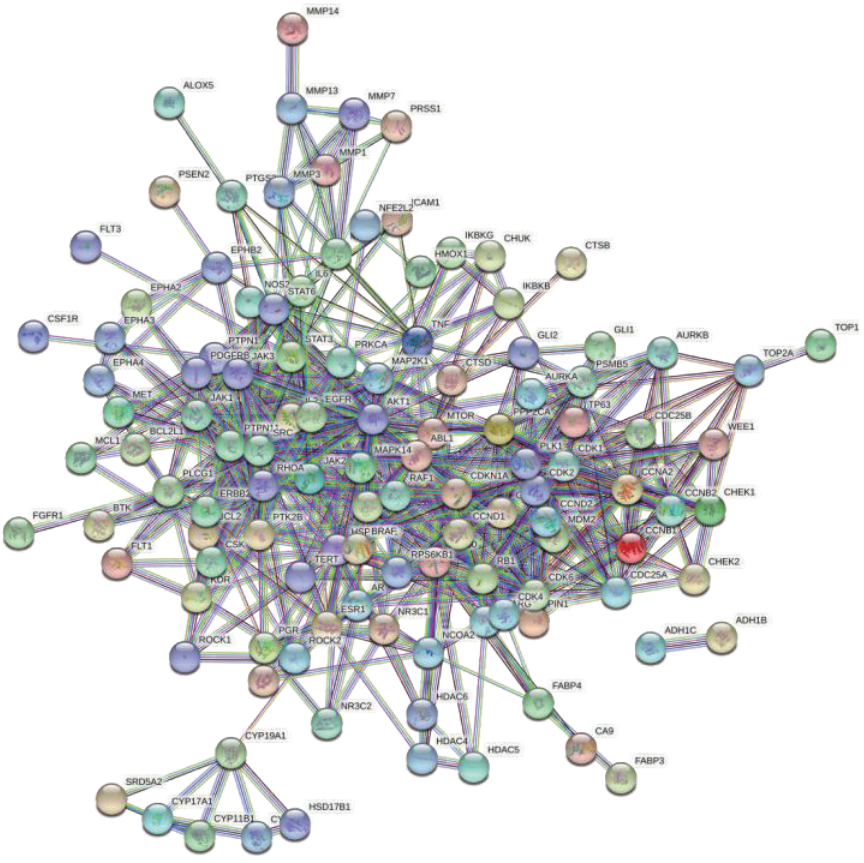


Fig. 3 Protein-protein interaction network diagram of CZ and BC

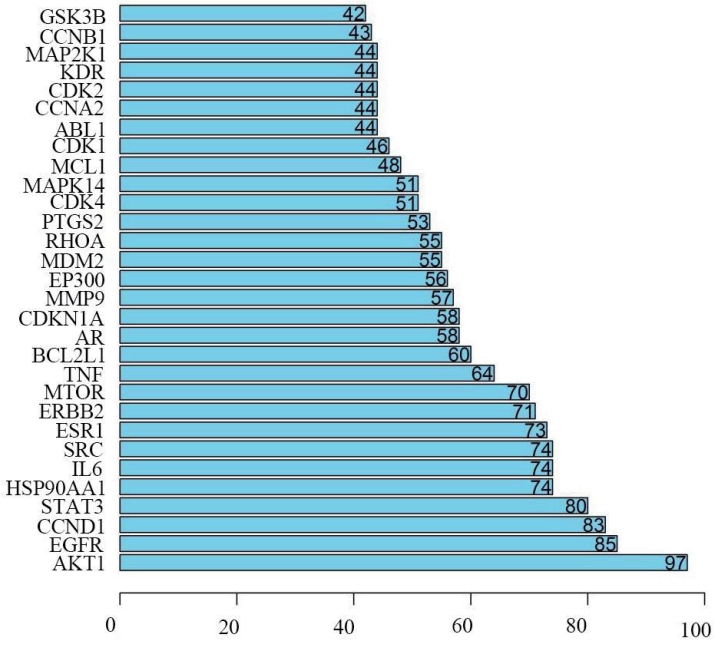


Fig. 4 Key gene diagram of CZ and BC

3.7 Curcuma anti-breast cancer GO and KEGG biological enrichment analysis

3.7.1 GO functional analysis

By using the R language to run the "clusterProfile" package, a total of 2,222 biological information entries were obtained through gene enrichment intersection. Gene Ontology (GO) analysis was performed on the top ten cellular components, molecular functions and biological processes respectively. Bar plots (Fig. 5) and bubble plots (Fig. 6) were generated, where redder colors indicate higher enrichment levels. Sixty-seven cellular components were involved in the GO enrichment analysis, including serine/threonine protein kinase complex, cyclin-dependent protein kinase holoenzyme complex and protein kinase complex. Among the 113 molecular functions, the top-ranked molecular functions are protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, and protein serine/threonine kinase activity. Through GO analysis, 2042 biological processes were obtained. The top ten biological processes in the anti-breast cancer mechanism of CZ were peptidyl-tyrosine phosphorylation, peptidyl-tyrosine modification, positive regulation of cell cycle, peptidyl-serine modification, peptidyl-serine phosphorylation, regulation of reactive oxygen, regulation of reactive oxygen, species metabolic process, response to antibiotic, response to reactive oxygen species, and positive regulation of protein serine/threonine kinase activity.

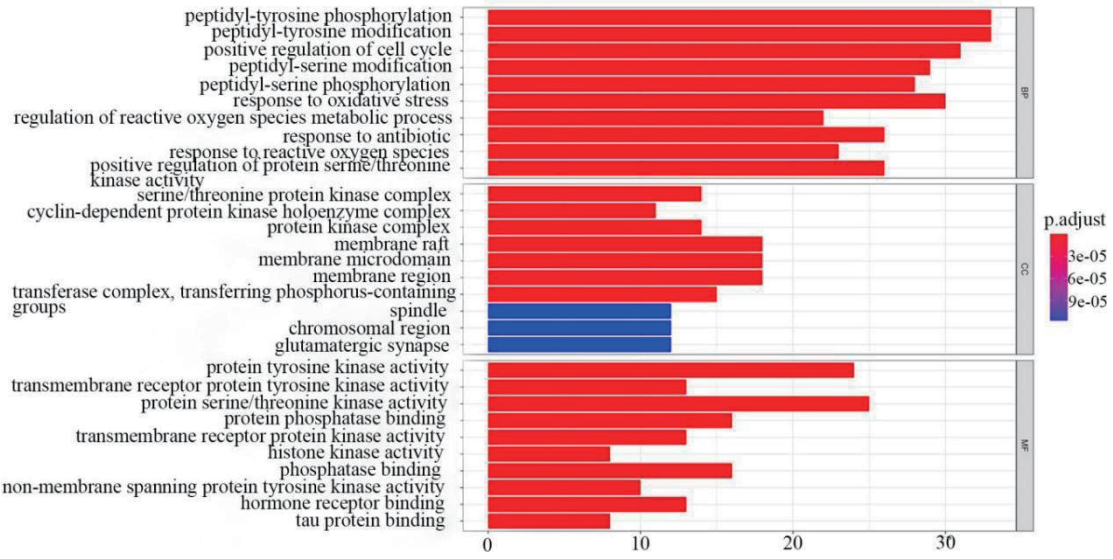


Fig. 5 GO analysis bar chart of *Curcuma zedoaria*'s anti-breast cancer effects

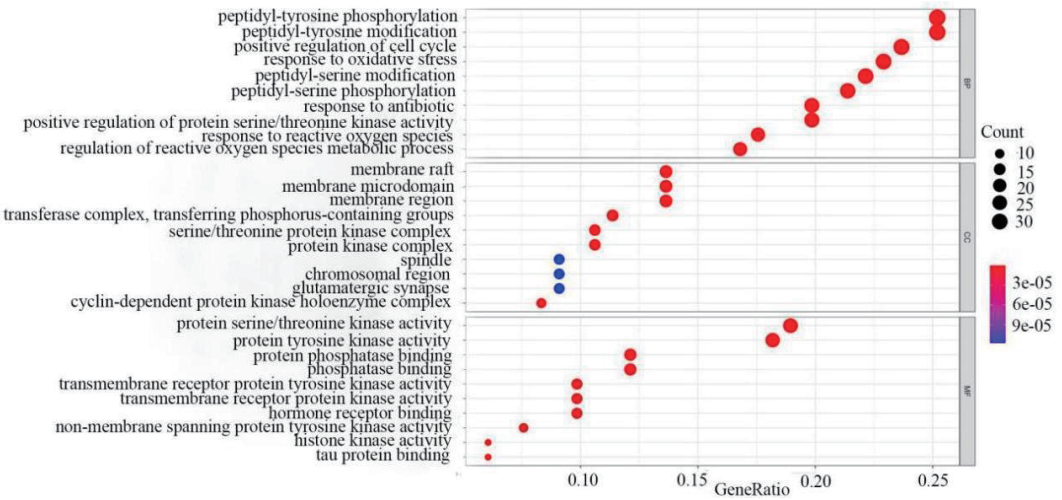


Fig. 6 Bubble plot of GO analysis for CZ against breast cancer

3.7.2 KEGG functional analysis

KEGG pathway enrichment analysis was performed with the "clusterProfiler" package in R language, with the screening criterion of $P < 0.005$. The top 20 significantly enriched pathways were selected for bar chart (Fig. 7) and bubble plot (Fig. 8) visualization, where the color depth represents the enrichment level (redder color indicates higher enrichment). The top ten enriched pathways were Prostate cancer, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, Hepatitis B, Pancreatic cancer, Human cytomegalovirus infection, MicroRNAs in cancer, Proteoglycans in cancer, Cell cycle, and Endocrine resistance. Most of these pathways are closely associated with the occurrence and development of BC, suggesting that CZ exerts its anti-BC effect through the synergistic regulation of multiple signaling pathways.

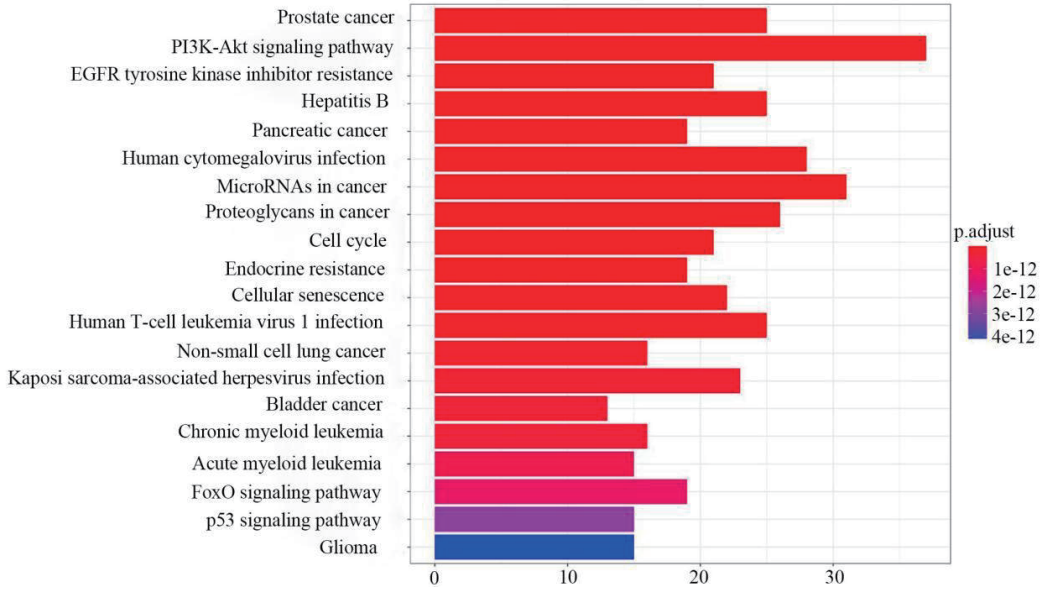


Fig. 7 KEGG analysis bar chart of CZ against breast cancer

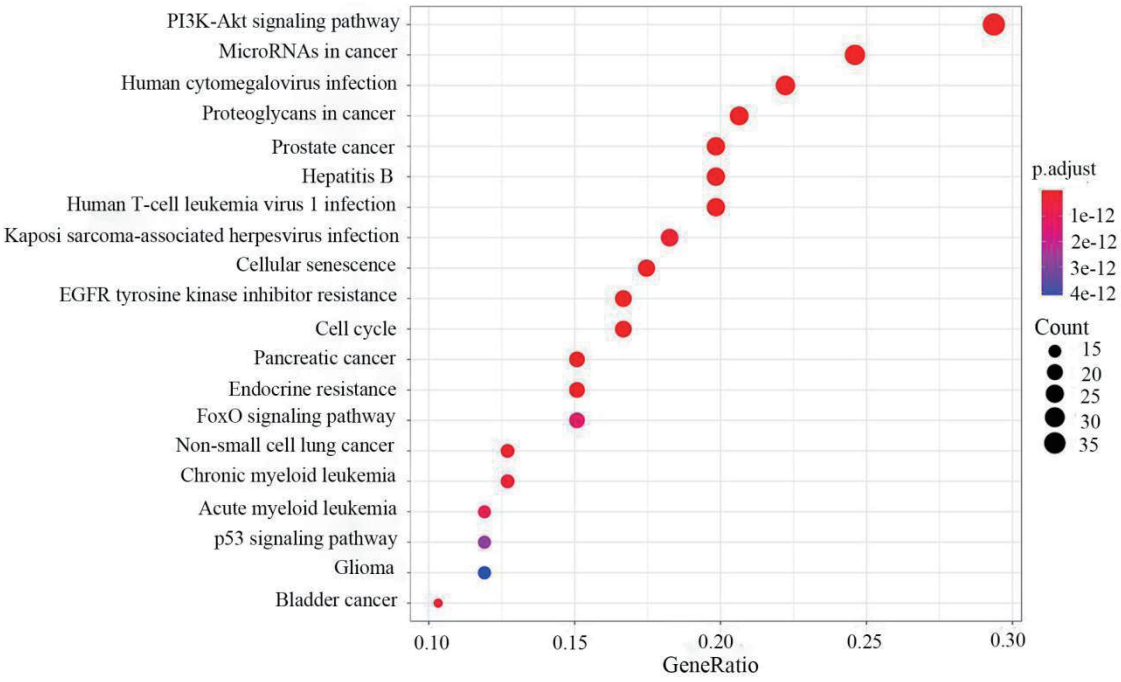


Fig. 8 CZ anti-breast cancer KEGG analysis Bubble plot

3.8 Molecular docking analysis

The top 5 active components of CZ (ranked by degree value) and top 6 core targets (AKT1, EGFR, CCND1, STAT3, HSP90AA1, IL6) were selected for molecular docking verification, resulting in 30 receptor-ligand pairs (Table 3, Fig. 9). The docking results showed that 27 pairs (90.0%) had a binding Affinity < -5 kcal/mol, and 3 pairs had an Affinity < -7 kcal/mol, indicating a good binding activity between CZ active components and core targets [19]. The highest binding affinity was observed in the hederagenin-HSP90AA1 complex (Affinity = -7.6858 kcal/mol), while the lowest was the curcumenone-EGFR complex (Affinity = -4.5050 kcal/mol). These results provide a reliable molecular basis for subsequent pharmacological research on CZ's anti-BC effect.

Table 3 The results of molecular docking

Components	Affinity (kcal/mol)					
	AKT1	EGFR	CCND1	STAT3	HSP90AA1	IL6
Bisdemethoxycurcumin	-6.3032	-6.5252	-6.1310	-6.3171	-6.5694	-5.6947
Curcumenone	-5.4319	-4.5050	-5.2082	-4.5915	-5.6600	-4.9426
Curcumin	-6.5120	-6.3775	-6.1754	-5.9980	-7.3026	-6.1286
Demethoxycurcumin	-5.9981	-5.8823	-6.1259	-5.8727	-7.3751	-5.4856
Hederagenin	-6.0009	-6.0131	-5.8986	-5.6964	-7.6858	-5.7074

Note: A: 3D structure of receptor & ligand, protein backbone shown as tubes, ligands in pink sticks; B: 2D interaction diagram, protein residues as circles colored by properties—green for hydrophobic, purple for polar.

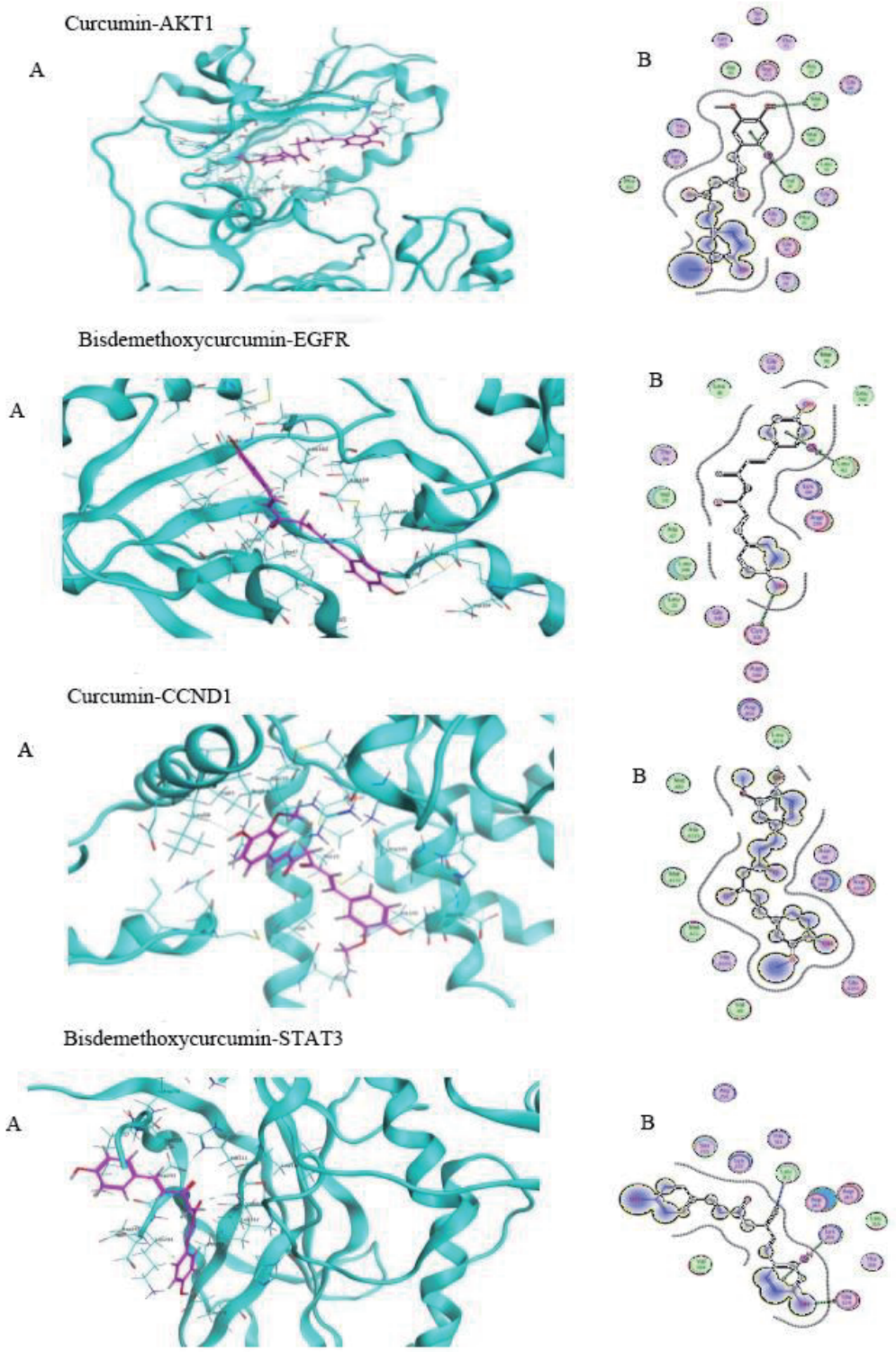


Fig. 9 Partial molecular docking diagram

4 Discussion

With the rapid development of network pharmacology in TCM research, its inherent characteristics of integrity and systematicness are highly consistent with the holistic concept and syndrome differentiation and treatment of TCM. By constructing the CZ-BC interaction network, the anti-tumor mechanism of CZ can be systematically elucidated, which reflects the transformation of pharmacological research from the traditional "single component-single target" model to a comprehensive network analysis model. At present, network pharmacology has been widely used in TCM research and has become an important bridge connecting TCM and modern disease research [12, 20].

In this study, six reported anti-cancer components (e.g., β-elemene, curcumin, and germacrone) were supplemented to the CZ active component library to ensure the comprehensiveness of the research. In the CZ component-BC-target network, germacrone, demethoxycurcumin, and bisdemethoxycurcumin (supplemented by literature mining) were located at the core of the network. These components were identified as the key active compounds of CZ against BC, which is consistent with the results of previous anti-BC studies on CZ. This indicates that the combination of database retrieval and literature mining can effectively screen potential anti-BC active components of CZ, and CZ can exert its anti-BC effect by targeting multiple oncogenic genes.

Through protein interaction analysis, AKT1, EGFR, CCND1, STAT3, HSP90AA1, IL6, SRC, ESR1, and ERBB2 were identified as key target genes of CZ in treating breast cancer. A theoretical breakthrough is conducive to the multi-targeted collaborative anti-tumor mechanism. AKT1 is a subtype of AKT and serves as an essential downstream target in the PI3K/AKT signaling pathway, regulating tumor growth, proliferation, apoptosis, survival, and the invasiveness of tumor cells. The occurrence of breast cancer is often manifested as alterations in the PI3K/AKT signaling pathway, and the formation of malignant tumors may be associated with the overexpression of AKT, which is involved in the growth of localized tumors [21-22]. Furthermore, AKT1 can regulate the cell cycle, reduce the impact of cell cycle inhibitors, thereby affect tumor growth [23]. Therefore, it can be inferred that CZ may exert its anti-BC effect by regulating AKT1, the key target of the PI3K/AKT signaling pathway, and further participating in the biological process of cell cycle regulation. CCND1 is a highly polymorphic oncogene closely associated with BC carcinogenesis. Mutation, amplification, and overexpression of CCND1 can disrupt cell cycle progression and abnormally activate cell cycle-related signaling pathways. Previous studies have shown that miR-93 can target CCND1 and E2F1, thereby inactivating the pRb/E2F1 pathway and inducing AKT phosphorylation, ultimately exerting an anti-BC effect [24]. The expression of CCND1 is related to EGFR. EGFR, as a member of the epidermal growth factor receptor family, is one of the primary oncogenes in breast cancer [25]. The PI3K-Akt signaling pathway can regulate the expression of cyclin D1 and can induce the growth of factor-dependent proliferation. Activating the downstream pathways of EGFR can promote the proliferation and differentiation of tumor cells, leading to tumor formation. The overexpression of EGFR is associated with tumor cell metastasis, invasion, and a poor prognosis. For instance, the over- expression of EGFR is related to the high malignancy and poor prognosis of triple-negative breast cancer. Therefore, EGFR can be regarded as a potential target for the treatment of breast cancer [26-27]. Additionally, IL-6 influences the growth, metastasis, proliferation, and apoptosis of breast cancer cells [28]. At the same time, the mTOR gene influences the proliferation of breast cancer cells [29], making

it a crucial therapeutic target for breast cancer treatment. Furthermore, we conducted a preliminary verification through molecular docking, and the results showed that there was a strong affinity between the ligand and the receptor.

GO functional analysis revealed that CZ exerts its anti-BC effect by regulating multiple cellular components and molecular functions involved in a variety of biological processes, including peptidyl-tyrosine phosphorylation/modification, positive regulation of cell cycle, peptidyl-serine phosphorylation/modification, oxidative stress response, and reactive oxygen species (ROS) metabolism and regulation. Previous studies have confirmed that germacrone can induce cell cycle arrest and apoptosis in BC cells, thereby inhibiting cell proliferation by regulating cell cycle progression [15], and daucosterol can inhibit cancer cell proliferation by inducing ROS-dependent autophagy [17]. These results suggest that CZ mainly exerts its anti-BC effect by regulating the biological processes of cell cycle and ROS metabolism.

KEGG pathway enrichment analysis identified the PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, MicroRNAs in cancer, Proteoglycans in cancer, and Cell cycle as the key signaling pathways through which CZ core targets regulate BC progression. miRNAs are essential regulatory factors in BC occurrence, development, metastasis, and chemoresistance, and have been recognized as potential therapeutic targets for BC [30]. miRNAs can interact with the core target genes (STAT3 and AKT1) of CZ and play a pivotal regulatory role in BC carcinogenesis. For example, Zhang et al. [31] found that miR-409-3p inhibits BC cell proliferation, migration, and invasion by downregulating AKT1; Deng et al. [32] confirmed that miR-519d overexpression targets STAT3 to inhibit BC cell proliferation and invasion and induce apoptosis; Zhao et al. [33] found that miR-21 negatively regulates STAT3 expression in MCF-7 BC cells, thereby suppressing cell invasion. Gene enrichment analysis revealed that genes such as AKT, MAPK1, EGF, and IL-6 can influence the PI3K/Akt signaling pathway to exert anti-cancer effects. The PI3K/Akt signaling pathway activates PI3K by promoting Akt phosphorylation, which regulates cell proliferation and thereby inhibits tumor growth. Zederone, as an active chemical component of CZ, affects the migration and invasion abilities of breast cancer cells by downregulating the phosphorylation level of AKT [34]. Therefore, through GO and KEGG enrichment analysis, the synergy of these drugs can, to some extent, overcome the problem of drug resistance caused by single-target drugs. This study further verifies the feasibility of using network pharmacology to explore the potential anti-BC mechanism of CZ.

In summary, this study used network pharmacology to systematically analyze the core targets, GO terms, and KEGG pathways of CZ against BC. The results demonstrated that key active components of CZ (e.g., demethoxycurcumin, germacrone, and bisdemethoxycurcumin) act on core target genes including AKT1, EGFR, CCND1, STAT3, HSP90AA1, and IL6, participate in multiple biological processes such as cell cycle regulation and ROS metabolism, and regulate key signaling pathways including the PI3K-Akt pathway, MicroRNAs in cancer, and Cell cycle, thereby exerting a comprehensive anti-BC effect. This study is only a preliminary exploration of the potential anti-BC mechanism of CZ, and many key genes and pathways identified in this study have not been verified by *in vitro* and *in vivo* experiments, thus requiring further in-depth research. This study lays a theoretical foundation for the subsequent pharmacological research on the anti-cancer effect of CZ, especially its anti-BC effect, and also provides a new idea for the development and clinical application of novel TCM-based targeted anti-cancer drugs.

5 Conclusion

This study explored the anti-BC mechanism of CZ using the network pharmacology approach. The results identified 23 bioactive components of CZ with anti-BC potential, 370 corresponding potential protein targets, and 1,766 BC-related target genes. CZ exerts its anti-BC effect by regulating 132 drug-disease overlapping genes. The construction of the CZ component-target network confirmed the "multi-component, multi-target" characteristic of CZ in BC treatment, which lays a foundation for the research on multi-target synergistic anti-tumor therapy. PPI network analysis identified the top ten core genes (e.g., AKT1 and EGFR), which play a pivotal role in the anti-BC effect of CZ. GO and KEGG enrichment analyses further revealed that CZ exerts its anti-BC effect through the synergistic regulation of multiple biological processes and signaling pathways. In conclusion, CZ exerts its anti-BC effect by acting on multiple core target genes and signaling pathways through its active components, thereby regulating the proliferation, apoptosis, migration, and invasion of BC cells. This study provides a theoretical basis for the clinical application of CZ in BC treatment and offers a new direction for the development of novel anti-tumor drugs based on TCM active components.

Acknowledgments

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