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Mechanisms of Honeysuckle Against Triple-negative Breast Cancer Based on Network Pharmacology and Molecular Docking

Kenian Pan [1] *Zhongren Gai* [2] *Tian Bo* [*]

Abstract: [Objective] To study the mechanism of action of the Honeysuckle against triple-negative breast cancer based on network pharmacology and molecular docking method, to provide a reference for elucidating its clinical anti-breast cancer mechanism. [Methods] The Honeysuckle active ingredients and their targets were screened by TCMSP and SwissTargetPrediction databases, and the target genes related to breast cancer were collected by GeneCards, OMIM and Disgenet databases, and the "Drug-Active Ingredient-Target-Disease" was constructed by using Cytoscape 3.9.1 software. The Cytoscape 3.9.1 software was used to construct a "drug-active ingredient-target-disease" network. The potential targets were imported into the STRING database to build a protein-protein interactions network (PPI). The core targets were annotated with the GO function and analyzed by KEGG pathway enrichment through the DAVID database. Molecular docking was carried out between the main active ingredients of the Honeysuckle and the potential targets through the Pymol software and AutoDock software. Molecular docking was performed using Pymol and AutoDock software. A total of 23 active ingredients and 591 targets of the

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Honeysuckle, 1,811 triple-negative breast cancer disease targets, 186 potential targets, and 38 genes in the PPI network were screened in the two-component target databases, TCMSP and SwissTargetPrediction, and the three disease databases, GeneCards, OMIM, and Disgenet. In the PPI network, 38 key targets of the honeysuckle for breast cancer treatment were obtained, and after GO and KEGG analysis, 1,118 GO analysis results and 157 KEGG pathways were obtained. The GO analysis results showed that the honeysuckle's anti-breast cancer may be related to the transcription initiation process of the RNA polymerase II promoter, nuclear chromatin, DNA binding, etc.; the KEGG analysis results showed that the honeysuckle may be involved in the cancer pathway and the anti-breast cancer may involve the cancer pathway. The results of the KEGG analysis indicated that the honeysuckle anti-breast cancer might be involved in the cancer pathway, proteoglycan signaling pathway, microRNA pathway, thyroid hormone signaling pathway, etc. The molecular docking results showed that the predicted key components, quercetin and lignocerol, had better binding properties to the core targets, EP000, ERBB2, GAPDH, and MAPK3. [Conclusion] The anti-triple-negative breast cancer of the Honeysuckle mainly involves EP000, ERBB2, GAPDH , MAPK3 and 157 signaling pathways, and its active ingredients can inhibit the growth of tumor cells through multi-targets, multi-pathways, and multi-mechanisms.

Keywords: **Honeysuckle, Triple-negative breast cancer, Network pharmacology, Quercetin, Lignans, Molecular docking**

1. Introduction

Breastcancer (CB) is the uncontrolled proliferation of breast epithelial cells in response to a variety of carcinogenic factors[1] CB is a phenomenon of uncontrolled proliferation of breast epithelial cells under the action of various carcinogenic factors. In the early stage of the disease, there are no obvious symptoms, but later on, it can be manifested as breast lumps, abnormal breast skin with "the orange peel sign" or "dimple sign", and nipple overflow in non-pregnant or breastfeeding state, etc. As the disease worsens, the cancer cells invade the surrounding lymphatic tissues and vital organs such as the liver and lungs, which seriously threaten the life and health of the patients. As the disease deteriorates, the cancer cells invade the surrounding lymphatic tissues and critical organs such as the liver and lungs, seriously threatening the life and health of patients. By 2020, the number of new cancer cases worldwide is estimated to increase. It is estimated that in 2020, there will be about 2.26 million new cases of breast cancer worldwide, and about 680,000 people will die of breast cancer; among them, 410,000 new cases of breast cancer will be found in China, accounting for about 1/5 of the world's cases, and the growth of breast cancer incidence rate in China has reached more than twice of the world's since 1990[2] As a result, the problems of high morbidity, mortality and poor quality of life are severed.

The clinical treatment of breast cancer includes surgery, endocrine therapy, adjuvant chemotherapy, radiation therapy, and targeted therapy. The clinical treatment of breast cancer includes surgery, endocrine therapy, adjuvant chemotherapy, radiotherapy, targeted therapy, etc. However, there are still many difficulties. The same stage of the disease and the same treatment methods, but the prognosis is very different, indicating the tumor's heterogeneity and different biological characteristics. Generally speaking, breast cancer treated by surgery has already developed to the middle stage, and the patient's physique is weak. Metastasis may have already occurred, and it is easy to recur after surgery, and once recurrence occurs, it will give the patient more psychological pressure and difficulty in treatment; at present, endocrine therapy is only suitable for some patients with hormone-receptor-positive breast cancer, and its therapeutic effect has a close relationship with the hormone-receptor-positive rate of expression. Its therapeutic cycle is longer, and it may even take five years. Its treatment effect is also closely related to the positive rate of hormone receptor expression, and the treatment period is extended, even five years, some even more than ten years or longer.[3] The disadvantages of adjuvant chemotherapy are even more obvious. Chemotherapy for breast cancer is very harmful to the body, and chemotherapeutic drugs will kill normal cells while killing cancer cells, which will lead to the lowering of white blood cells, bone marrow suppression, nausea and vomiting, weight loss, and many other complications[4] The survival rate after treatment is still unsatisfactory and cannot reach the ideal level. Early diagnosis is still the primary mean to prevent breast cancer from becoming malignant. Still, due to the influence of many reasons, the detection rate of carcinoma in situ in our country is relatively low, and most of the diagnosed breast cancers develop more rapidly.

Honeysuckle is also known as Lonicera japonica. Lonicera japonica for short baths is called golden flower, silver flower, heron duck flower, and so on. According to our traditional medicine, the honeysuckle is sweet and cold, attributed to the lungs, heart, and stomach, and is rich in medicinal components, including flavonoids, volatile oils, terpenoids, and glycosides, etc., which are considered to have the efficacy of clearing heat and removing toxins, detoxifying the epidermis and dispersing the wind, and reducing swelling, sterilization and anti-inflammatory effects by the traditional Chinese medicine. In recent years, the literature has reported that the honeysuckle and its chemical constituents can promote apoptosis in liver, lung, and gastric cancers and ameliorate cancer.[5]

In this study, the network pharmacological evaluation of the honeysuckle absorption, distribution, metabolism, and excretion (ADME) was performed, the active ingredient and the target for the treatment of triple-negative breast cancer were screened, and the target-enriched functions and pathways were analyzed. The effective components, targets, and mechanism of action of the honeysuckle were investigated. The mechanism of action of honeysuckle in the

treatment of diseases will provide a theoretical basis and new opportunities for developing and applying the honeysuckle in treating triple-negative breast cancer.

2. Methodology

2.1 Screening of major active components and targets of Honeysuckle (Honeysuckle)

The TCMSP (Https: // tcmspw.com/tcmsp.php) was used to search the keyword "honeysuckle" to obtain the active ingredients and their corresponding targets, and the screening conditions were set as Oralbioavailability (OB) ≥30%, Druglikeness (DL) ≥0.18, and the screening conditions were set as Oralbioavailability (OB) ≥30%, Druglikeness (DL) ≥0.18, and screening conditions were set as Oralbioavailability (OB) ≥0.18. The screening conditions were oral bioavailability (OB) ≥30% and Druglikeness (DL) ≥0.18, and the active ingredients of the Honeysuckle were collected and supplemented through the review of related literature. After obtaining the target ingredient, the target protein name of the active ingredient was obtained at "Related Targets", and the CAS number of the chemical ingredient without target information was obtained from the Pubchem database (Https://pubchem.ncbi.nlm.nih.gov/). Canonical SMILES, using the Swiss Target Prediction database (Http://www.swisstargetprediction.ch/) to predict the target informations, by setting Probability* >0 as our selection object. The collected target names were standardized and corrected through the Uniprot platform (Https: // www.uniprot.org/) with the filter condition: "organism: homo sapiens".

2.2 Breast Cancer Disease Target Screening

The target genes of "breast cancer" were searched in the OMIM database (Https://omim.org/), Genecard database (Https://www.genecards.org/), and Disgenet database (Https://www.disgenet.org/home/). The target genes of "breast cancer" were combined and de-replicated to obtain the relevant disease targets of breast cancer.

2.3 Active ingredient-disease shared target screening

The collected breast cancer related disease targets and honeysuckle active ingredient related targets were imported into the software Venny2.1 (Https://bioinfogp.cnb.csic.es/tools/venny/) for analysis, screened to obtain the intersection to get the potential action targets of the honeysuckle in treating breast cancer, and plotted the Venn Venn diagram.

2.4 Construction of Protein Interaction Networks (PPIs)

The potential targets of the Honeysuckle for breast cancer treatment obtained in 1.3 were imported into the String database, and the species was selected as "Homosapiens" , the protein interaction network was constructed, the results were saved, and the raw data were processed to create a network file and a type file, respectively. The network and type files were imported into Cytoscape 3.9.1 software to draw the protein interaction network and set and adjust the parameters. Then CentiScaPe2.2 was used to calculate the Degree value, Betweenness value and Closeness value, which were calculated by the following formula in Excel: "Degree ＞ 40", "Betweenness

value" and "Closeness value". "Degree ＞ 40.5, BetweennessunDir ＞ 155.5, ClosenessunDir ＞ 0.003" were used as the conditions to screen the key targets, and the protein-protein interaction network and core target network were finally obtained.

2.5 GO and KEGG pathway enrichment analysis

The key targets obtained in 1.4 were imported into the DAVID database (https://david.ncifcrf.gov/) , and then clicked "Official-Gene-Symbol" and "GeneList". Click "Official-Gene-Symbol" and "GeneList", and select "Homosapicns" for the species. After uploading the data, KEGG pathway enrichment analysis was performed, and GO analysis was performed in three aspects: biological process (BP), cellular component (CC), and molecular function (MF). The difference was statistically significant. The data were exported to Excel and then imported into the Microbiology website (http: //www.bioin-formatics.com.cn/) for graphing to obtain GO analysis bubble diagrams and KEGG pathway analysis bubble diagrams.

2.6 "Drug-Active Ingredient-Target-Disease" Network Construction

The drug-active ingredient-target network in Honeysuckle was integrated with the results of KEGG-enriched metabolic pathways, and a "drug-active ingredient-target-disease-pathway" network diagram was constructed in Cytoscape 3.9.1. Each node in the network represents the active ingredient and the key target gene; edges are used to connect the active ingredient and the key target gene; nodes connected to the network are expressed in degrees. The greater the degree of the node in the network, the stronger the effect of the active ingredient.

2.7 Molecular Docking Validation

The top four targets in the PPI network were used as receptors, and the top two active ingredients of the Honeysuckle in the "active ingredient-target" network were used as ligands to predict the binding ability of the two, and the lower the value of binding energy, the better the binding ability. Uniprot queried the protein numbers corresponding to the first four core genes, and the protein crystal complexes were imported into the pymol software (https://www.rcsb.org/) for dewatering and ligand removal, and then hydrogenated by AntoDock software and exported to the pdbqt file; the 2D structure of the active ingredient of the Honeysuckle was downloaded from PubChem and saved as the pdbqt file. 2D structure of the active ingredient and saved as the sdf format through PubChem, converted to pdb format file through OpenBabel, pre-processing such as detection of torsion bonds and centers using AutoDock software and exported as the pdbqt file; docking of small-molecule ligands and protein crystal complexes using Autodockvina software and recording of binding free energies to analyze the degree of binding of the active ingredient to the target protein and to analyze the results of the binding degree of the active ingredient to the target protein. The free energy of binding was recorded to analyze the degree of binding between the active ingredient and the target protein, and Pymol visualized the analysis results.

3. Results

3.1 Screening of active ingredients and targets of tulip gold

A total of 236 chemical components of the honeysuckle were obtained from the TCMSP database, and 23 active components in the honeysuckle that the human body could absorb were screened by taking bioavailability OB ≥ 30% and drug-like DL ≥ 0.18% as the qualifying conditions (see Table 1). Among the 23 components, the quercetin and the lignocerol have higher contents in honeysuckle, and many of the traditional Chinese medicines in which they are found have anticancer effects; therefore, we selected the quercetin and lignans to explore their mechanisms of action against triple-negative breast cancer. At the same time, the target proteins corresponding to the active ingredients of the honeysuckle were extracted from the TCMSP database, and the target protein names were converted to gene names in the Uniprot database, and a total of 592 target genes were obtained.

Table 1 Effective the components of the Honeysuckle

	MolID	MoleculeName	OB (%)	DL
1	MOL001494	Mandenol	42	0.19
2	MOL001495	Ethyllinolenate	46.1	0.2
3	MOL002707	phytofluene	43.18	0.5
4	MOL002914	Eriodyctiol (flavanone)	41.35	0.24
5	MOL003006	(-)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano[4,3-d]oxazolo[3,2-a] pyridine-3-carboxylicacid_qt	87.47	0.23
6	MOL003014	secologanicdibutylacetal_qt	53.65	0.29
7	MOL002773	beta-carotene	37.18	0.58
8	MOL003036	ZINC03978781	43.83	0.76
9	MOL003044	Chryseriol	35.85	0.27
10	MOL003059	kryptoxanthin	47.25	0.57
11	MOL003062	4,5'-Retro-.beta.,.beta.-Carotene-3,3'-dio	31.22	0.55

		ne, 4',5'-didehydro-		
12	MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	51.96	0.41
13	MOL003101	7-epi-Vogeloside	46.13	0.58
14	MOL003108	CaerulosideC	55.64	0.73
15	MOL003111	Centauroside_qt	55.79	0.5
16	MOL003117	IoniceracetalidesB_qt	61.19	0.19
17	MOL003124	XYLOSTOSIDINE	43.17	0.64
18	MOL003128	dinethylsecologanoside	48.46	0.48
19	MOL000358	beta-sitosterol	36.91	0.75
20	MOL000422	kaempferol	41.88	0.24
21	MOL000449	Stigmasterol	43.83	0.76
22	MOL000006	luteolin	36.16	0.25
23	MOL000098	quercetin	46.43	0.28

3.2 The triple-negative breast cancer disease target screening

The 5193 genes related to triple-negative breast cancer were retrieved from the Genecards database, and 1299 results were taken after the median screening, 502 genes related to triple-negative breast cancer were retrieved from OMIM, and 245 genes related to triple-negative breast cancer were retrieved from Disgenet. The three databases of the triple-negative breast cancer related target using the online software Venny2.1 genes were taken and set together. Duplicates were removed to summarize 1811 target genes for triple-negative breast cancer (see Figure 1).

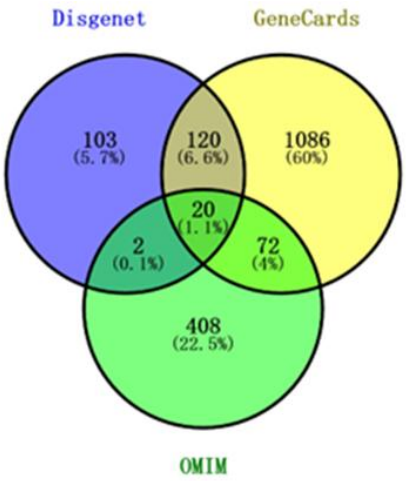


Figure 1 Wayne diagram of targets associated with triple-negative breast cancer

3.3 Screening of active ingredient-disease shared targets

The Venny2.1 tool was utilized to obtain 186 targets for the intersection of the honeysuckle active ingredient targets with triple-negative breast cancer disease targets (see Figure 2), i.e., honeysuckle may synergize in treating breast cancer through multiple potential action targets.

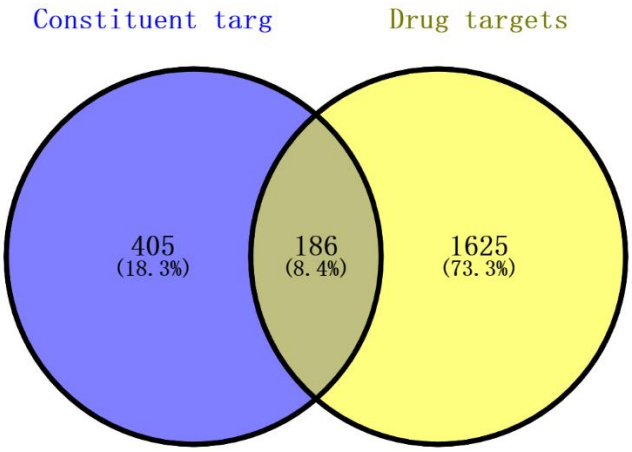


Figure 2 Wayne's diagram of targets related to active ingredients of the honeysuckle and targets related to triple-negative breast cancer

3.4 Protein Interaction Network (PPI) Construction

The obtained 186 intersecting target genes were imported into the String database to draw the protein interaction network (see Fig. 3), and the PPI network contained 185 nodes and 3743 edges, where nodes denote the target genes, and edges denote the interactions between the target genes. Through the obtained data, after filtering according to the conditions degree>40.5, Betweenness>155.5, Closeness>0.003, the obtained data were visualized and analyzed by Cytoscape 3.9.1 software to draw the core target network (see Figures 4-7), which contained 38

nodes and 610 edges, the color from dark to light indicates that the degree value of the node is from large to small, and the node from large to small indicates that the degree value of the node is from large to small. The four most core targets were screened according to the node values, which were the key targets of E1Abindingproteinp300 (EP300), tyrosine kinase receptor 2 (ERBB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and mitogen-activated protein kinase (MAPK3), and it was concluded that these targets played a key role in honeysuckle treatment of breast cancer.

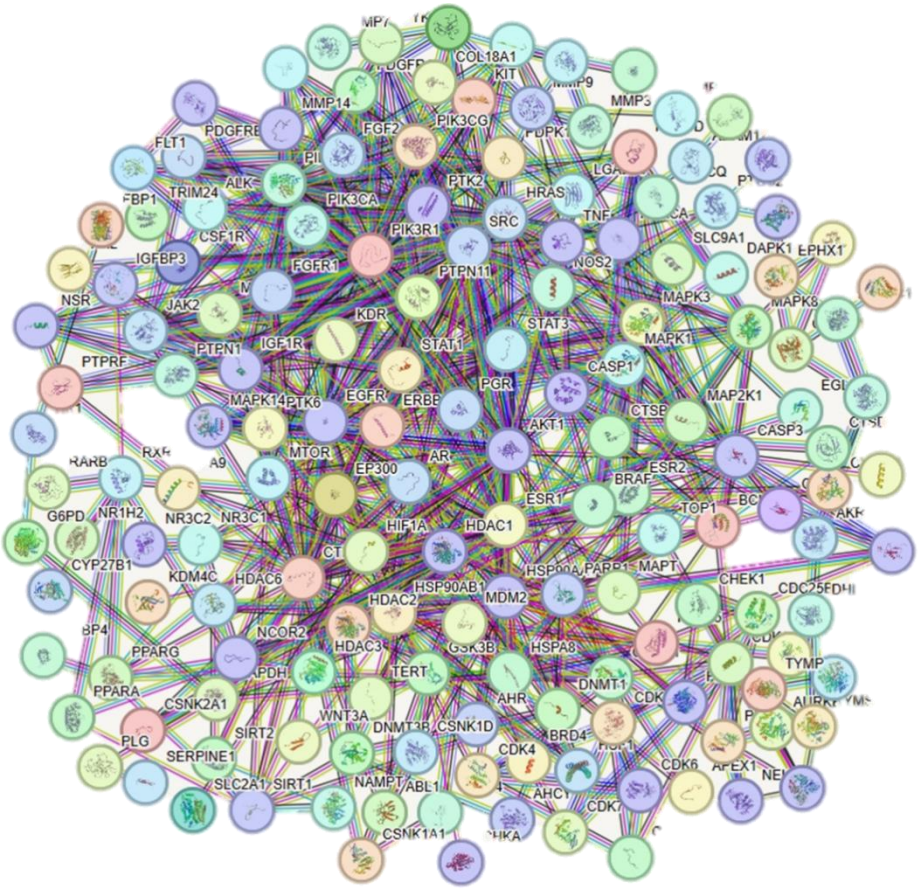


Figure 3 Target protein interaction network diagram

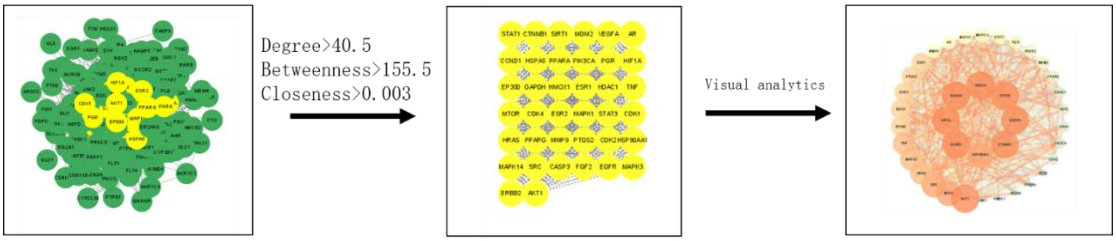


Figure 4 Visualization of PPI core target network

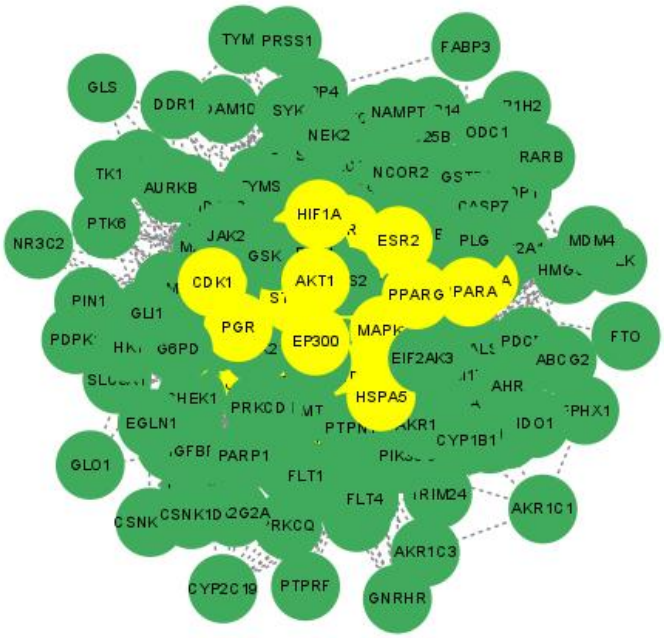


Figure 5 Potential target screening

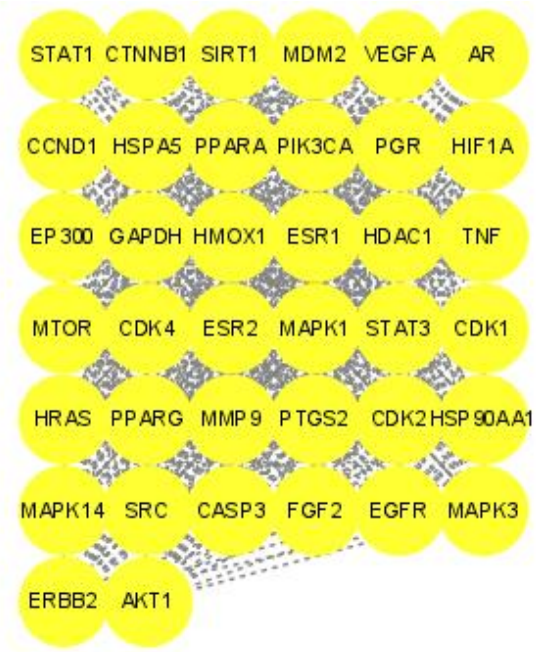


Figure 6 Potential targets

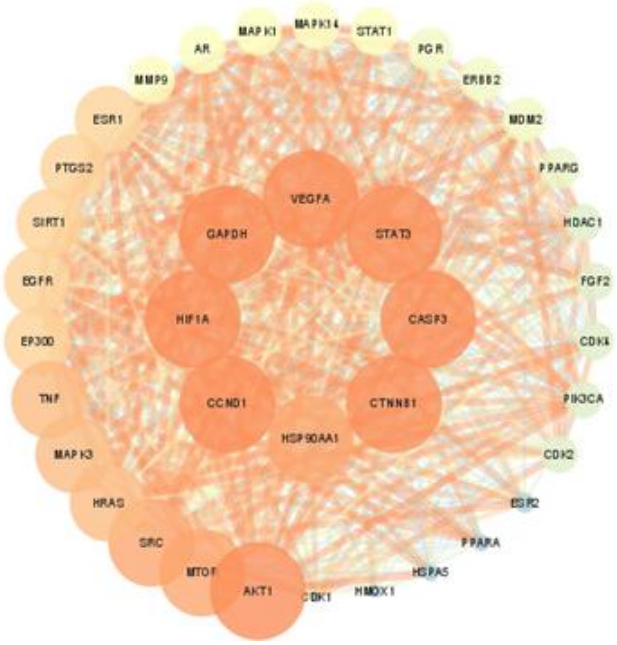
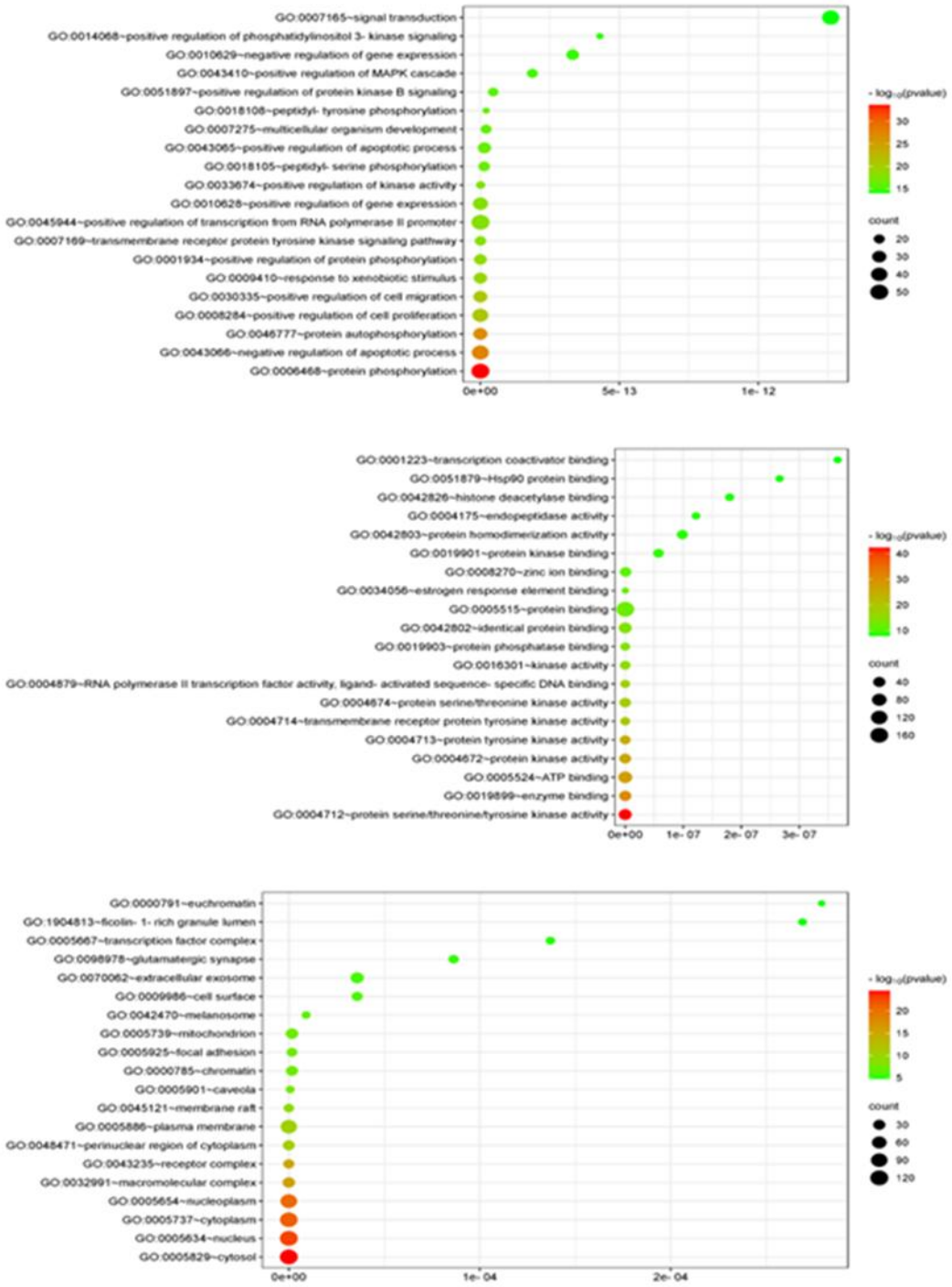


Figure 7 Core targets

3.5 Results of GO Functional Analysis

GO function analysis was performed using the DAVID database, resulting in 1118 biological processes, of which 833 were Biologicalprocess (BP), mainly involving protein autophosphorylation, positive regulation of kinase activity, negative regulation of the apoptotic process, peptidyl tyrosine phosphorylation, protein phosphorylation, etc.; 102 were Molecularfunction (MF), mainly involving receptor complexes, nucleoplasm, nucleus,

macromolecular complexes, plasma membrane, etc.; and Cellcomponent (CC) 102, mainly related to receptor complexes, nucleoplasm, nucleus, polymer complexes, plasma membrane, etc.; Molecularfunction (MF) 183, mainly related to transmembrane receptor protein tyrosine kinase activity, adenosine triphosphate (ATP) binding, protein phosphorylation, and protein phosphorylation. ATP) binding, protein tyrosine kinase activity, protein kinase activity, and identical protein binding. The top 20 entries of BP, CC, and MF were selected for bubble plot presentation (see Figure 8), suggesting that the honeysuckle acts on triple-negative breast cancer by modulating multiple biological processes.



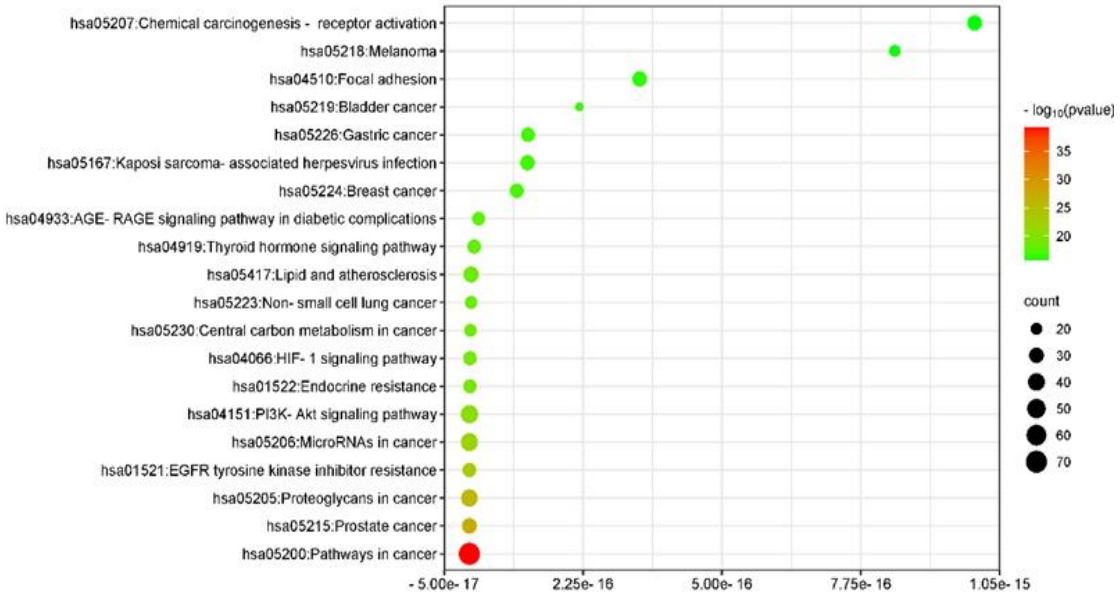
Where the horizontal axis is the enrichment factor and the vertical axis is the GO functional name

Fig. 8 Bubble plot of GO functional analysis results of honeysuckle treatment for the triple negative breast cancer

3.6 KEGG pathway enrichment analysis

The 157 signaling pathways were screened in the KEGG pathway enrichment analysis, and the top 20 pathways were taken for KEGG visualization to draw a bubble map (see Figure 9). The

major pathways associated with triple-negative breast cancer are the cancer pathway, proteoglycan signaling pathway in cancer, microRNA pathway, and thyroid hormone signaling pathway. The results suggest that the honeysuckle exerts its therapeutic effect on triple-negative breast cancer by acting on multiple signaling pathways.



Where the horizontal axis is the enrichment factor and the vertical axis is the pathway name.

Figure 9 Bubble plot of KEGG pathway enrichment analysis results of goldenseal treatment for triple-negative breast cancer

3.7 "Drug-component-target-disease-pathway" network construction

Cytoscape 3.9.1 software was used to construct a "drug-component-target-disease-pathway" network graph (see Figure 10), which had 231 nodes, including 23 components (green circles), 186 targets (blue quadrangles), 20 pathways (orange circles), and 854 edges. The graph predicts the number of associations between nodes using the degree value (the degree), and a larger degree value indicates that the component or target is more important, the top two components with a degree value of 49 are respectivel. The top two components with a degree value of 49 are Quercetin (Quercetin) with a degree value of 49 and the top two components with a degree value of 47 are (Quercetin) with a degree of 49 and luteolin (luteolin) with a degree of 47.

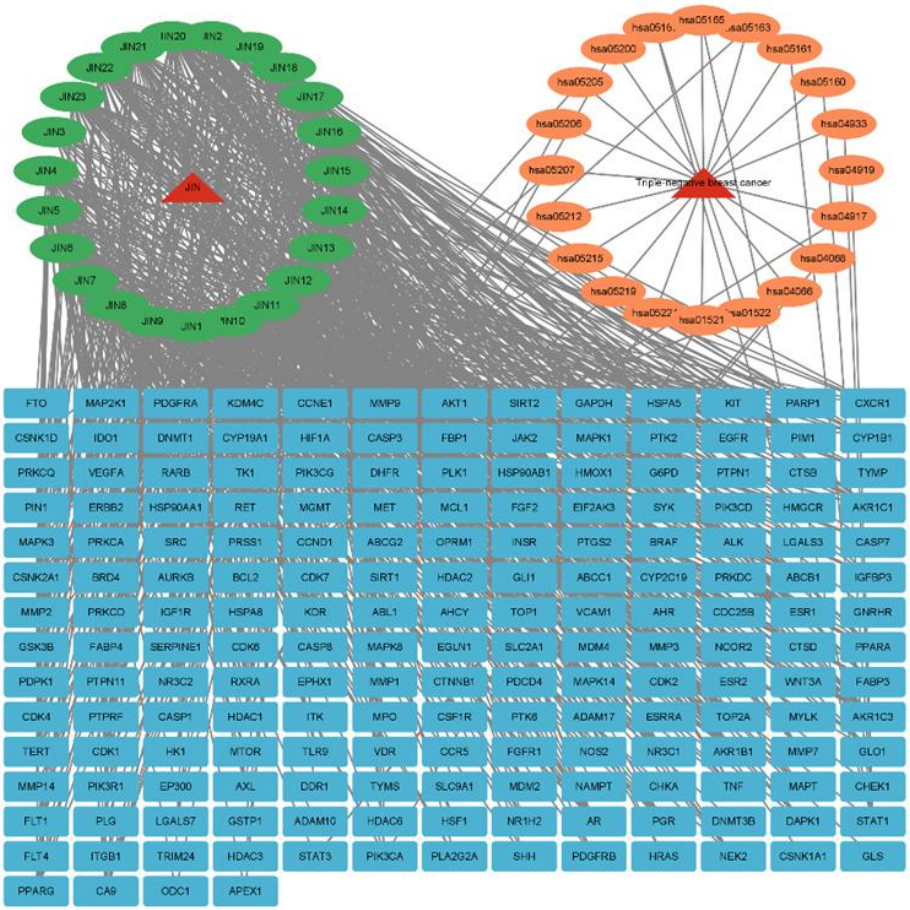
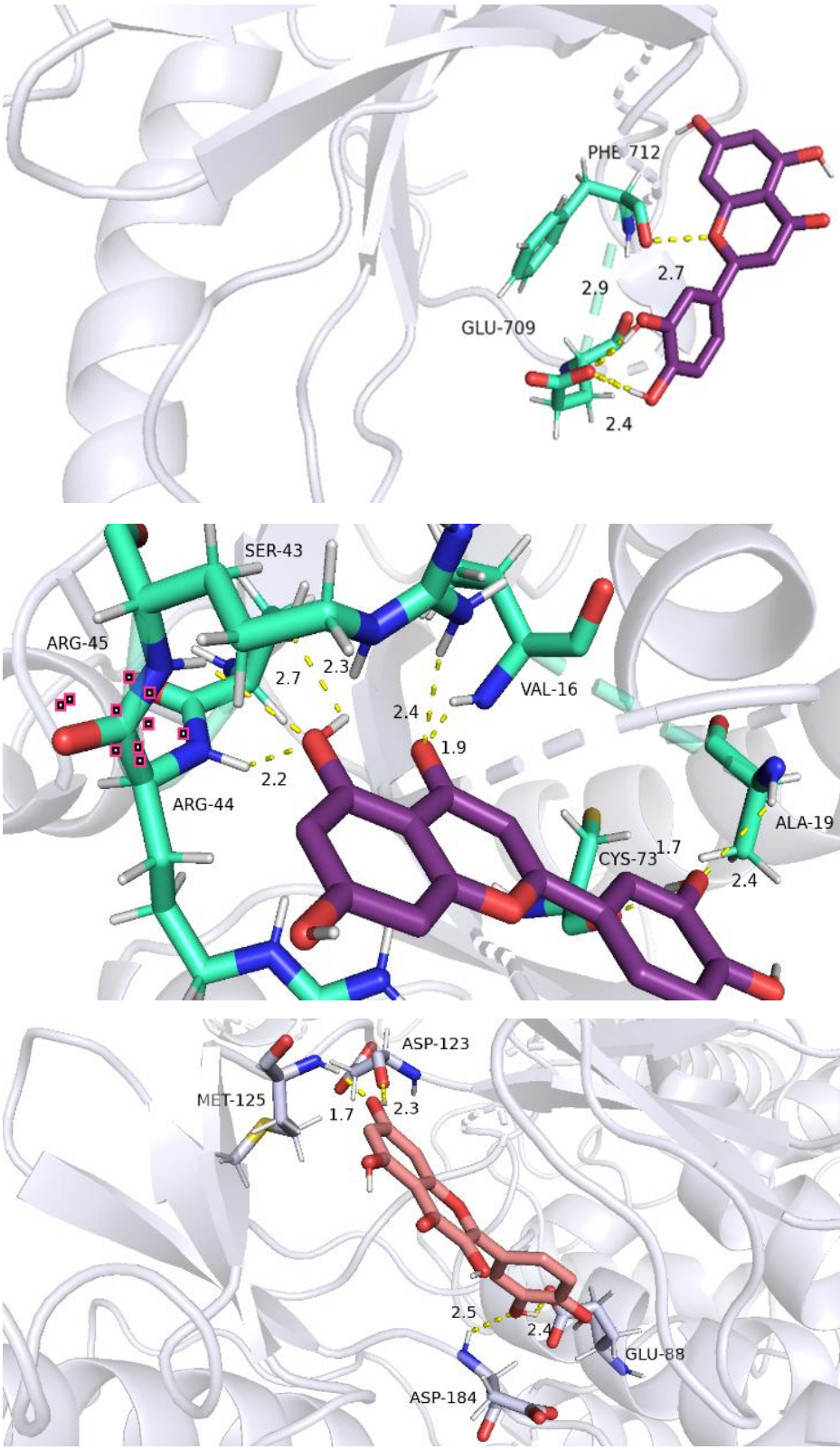


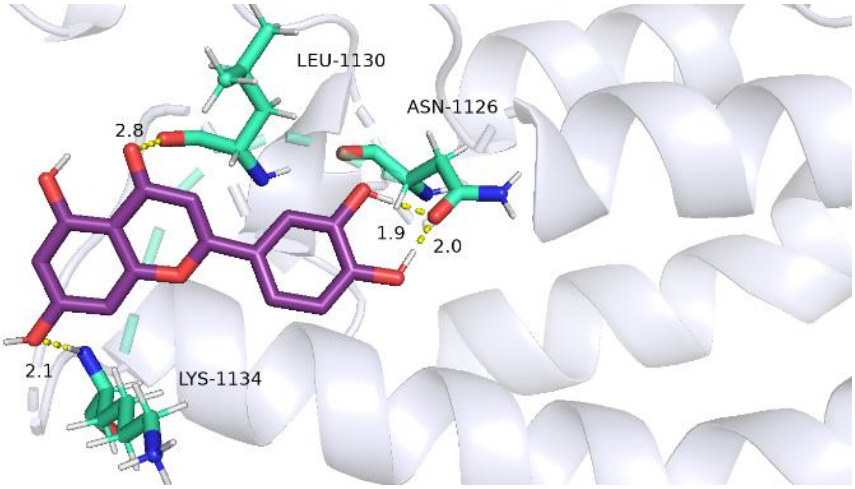
Figure 10 Drug-component-target-disease-pathway (DCP) diagram of Honeysuckle against triple-negative breast cancer.

3.8 Molecular docking validation

In this study, we compared the top-ranked targets in the PPI network, EP000, ERBB2, GAPDH and MAPK3, with the top-ranked targets in the "Active Ingredient Targets" network diagram, Quercetin, and the top-ranked targets in the "Active Ingredient Targets" network diagram. The binding ability of target proteins was predicted with target proteins as receptors and active ingredients as ligands, and the lowest binding energy was used as the result of docking between target proteins and ligands, which was analyzed and visualized using Pymol software (see Figure 11). If the binding energy is <-5.0 kcal/mol, it indicates that the target protein binds well to the active ingredient and the smaller the binding energy, the better the docking between the two. Based on the docking results, it can be seen that the binding energies of both components and targets were less than zero, indicating that most of the components and targets had good binding activities. Among them, ERBB2 and lignan have the lowest binding energy of -7.04 kcal/mol and the best binding ability; GAPDH and lignans The binding energy of GAPDH and lignocaine was the same as that of MAPK3 and Quercetin The binding energies of GAPDH and lignans were

lower than -5.0kcal/mol, which was the best binding ability; the study confirmed that the core targets of Honeysuckle were well docked with the corresponding active ingredients.

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2.8
1.9
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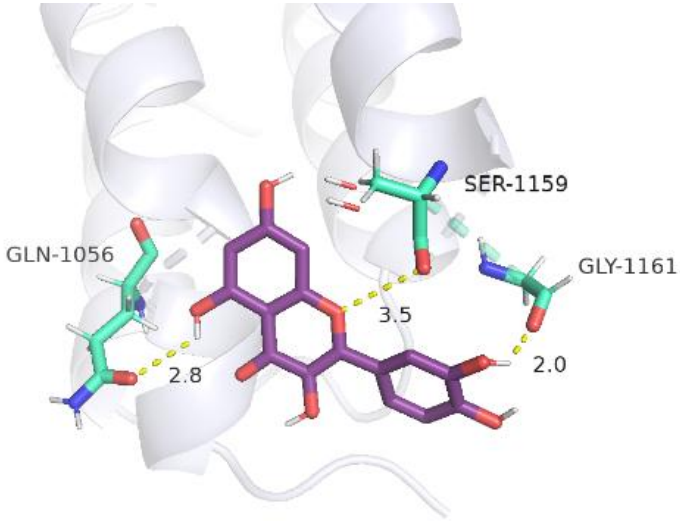
LYS-1134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3.5
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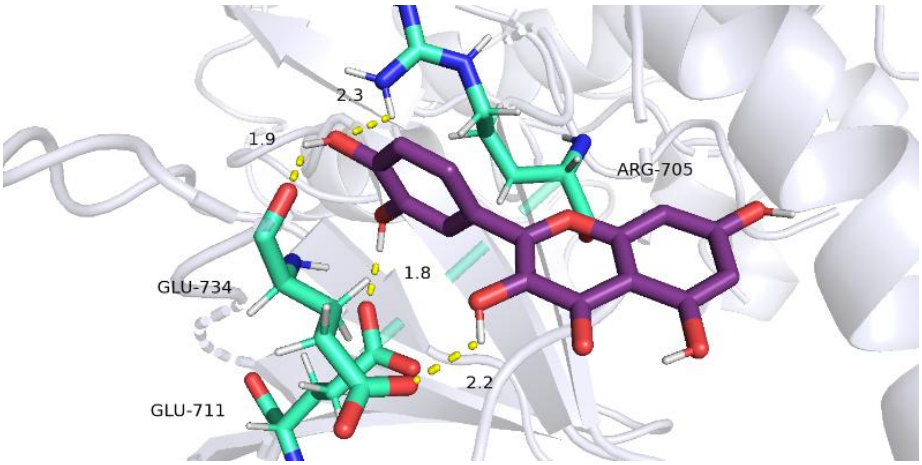
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ARG-705
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GLU-734
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GLU-711

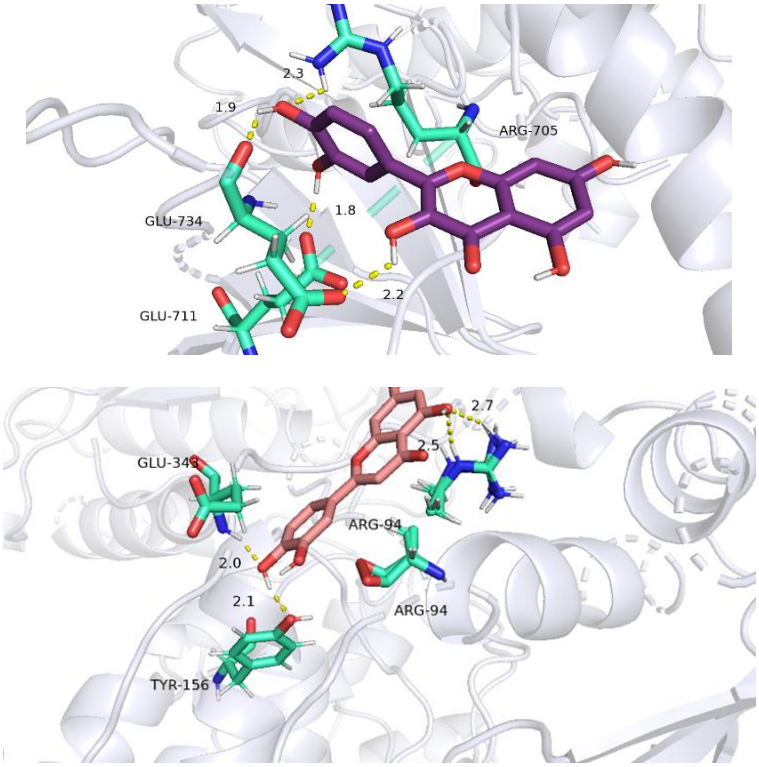

Figure 11 Visualization and analysis of molecular docking results

4. Discussion

Breast cancer is a highly heterogeneous disease with increasing morbidity and mortality rates year after year, posing a serious threat to patients' lives, health, and quality of survival. Breast cancer belongs to the category of "breast rock" in Chinese medicine. Breast cancer belongs to the category of "breast rock" in Chinese medicine. Ge Zhi Yu Lun" cloud: "If the husband is not in the husband, not in the aunt and uncle, worry and anger and depression, xin xi cumulative, the spleen qi obstruction, liver qi horizontal reversal, so into hidden nuclear name is milk rock."[7] The Surgical Zen" said: "melancholy injures the liver, thinking injures the spleen, accumulation of thoughts in the heart, the wish is not desired, to the meridian plagued and astringent, aggregated into a nucleus name is milk rock."[9] Emotional disorders, liver depression and spleen deficiency are the core etiology of breast cancer, and contemporary Chinese medicine practitioners also follow this theory, taking liver and spleen strengthening as the basic treatment for breast cancer.[10] Contemporary Chinese medicine also follows this theory, and takes detoxifying the liver and strengthening the spleen as the basic treatment for breast cancer. In China, Chinese medicine therapy has become one of the important means of breast cancer treatment. Honeysuckle has anti-inflammatory, antibacterial, immunomodulatory and other pharmacological activities.[11] It has been shown that the active components of the honeysuckle, Quercetin and lignans, can play an anti-tumor role by regulating a variety of cell signaling pathway, and are closely related to many

important molecular targets, such as cell growth, differentiation, apoptosis, and so on. However, during the TCM pharmacology study, we could not identify the mechanism of action due to the complexity of TCM components. The emergence of network pharmacology provides a new method for the study of single and formulated Chinese medicines. Compared with traditional screening experiments, network pharmacology is characterized by high efficiency and purposefulness, significantly reducing ineffective and blind pre-experiments. The results of network pharmacology are summarized in the following table.

In this study, based on the network pharmacology method, a total of 23 effective active ingredients such as the Quercetin and the lignans were screened by TCMSP database analysis, and the intersections were obtained by mapping the mined honeysuckle active ingredient-related targets with triple-negative breast cancer disease targets imported into the software VENNY 2.1.0,meanwhile the PPI network analysis showed a close connection between these intersected targets. It has been shown that both the Quercetin and lignans have pharmacological effects such as inhibiting tumor cell proliferation and inducing tumor cell apoptosis[13]. Lignans are natural flavonoids[14]. The inhibition of the expression of pro-oncogenic proteins, tumor size reduction, growth viability, progesterone-independent VGF secretion, and increase of Bax expression are promising mechanistic pathways for it. Lignans can inhibit proliferation and suppress the expression of p-STAT3, p-EGFR, p-Akt, p-Erk1/2 in EGF-induced MCF-7 breast cancer cells. It also inhibited the EGF-induced EGFR signaling pathway activity in human breast cancer cell lines. Quercetin is the main representative of the flavonol flavonoid subclass with a high potential for chemopreventive effects, demonstrated in in vitro and in vivo models. The anticancer effects of quercetin are dependent on its ability to reduce proliferation, induce apoptosis, lead to cell cycle arrest, and inhibit the mitotic process through the regulation of the cell cycle proteins, pro-apoptotic, PI3K/Akt, and mitogen-activated protein kinase (MAPK) molecular pathways ability to[17] . This suggests that the Honeysuckle may exert the antitumor effects as well as its active ingredients, the quercetin and lignans.

The network diagram of "drug-component-target-disease-pathway" shows that there are 186 targets of honeysuckle-triple-negative breast cancer after taking the intersection of honeysuckle-triple-negative breast cancer targets, and the target proteins of honeysuckle active ingredients for the treatment of triple-negative breast cancer, such as EP000, ERBB2, GAPDH and MAPK3, were found by further screening, which revealed the material basis and molecular mechanism of honeysuckle-triple-negative breast cancer resistance. ERBB2 is an important tyrosine kinase receptor, a member of the ErbB family, and it is active in the treatment of triple-negative breast cancer by interacting with other receptors in the family, including ERBB2, GAPDH, MAPK3, and EP000, which transmits signals downstream through heterodimerization

with other receptors in the family. It plays a vital role in various cellular responses, such as proliferation, differentiation, and adhesion. And ERBB2 is amplified at the DNA level in breast cancer (20%-30%); GAPDH is highly expressed as the rate-limiting enzyme of the serine synthesis pathway in some normal tissues and is particularly important in developing the nervous system[19].In vitro and in vivo experiments demonstrated that inhibition of GAPDH expression in breast cancer cells resulted in decreased cell proliferation, reduced serine synthesis, decreased tricarboxylic acid cycle intermediate α-ketoglutarate levels, and tumor growth inhibition. Breast cancer cell survival may depend on increased serine synthesis flux caused by GAPDH overexpression. Activated MAPK3 promotes apoptosis and malignant transformation by activating the stress-activated protein kinase system[20] . Related studies have found that MAPK3 can also be expressed in cancer tissues[21].GO enrichment analysis showed that the target genes of Lonchocarpus indicus against breast cancer are involved in the positive regulation of kinase activity, the activity of binding protein serine/threonine kinase, the positive regulation of MAPK cascade, the positive regulation of PI3K signaling, ATP binding, and other biological processes, which are important in the treatment of breast cancer by Lonchocarpus indicus. Through KEGG pathway enrichment analysis, a total of 157 pathways of honeysuckle against triple-negative breast cancer were found, mainly the cancer pathway, the proteoglycan signaling pathway in cancer, the microRNA pathway in cancer, and thyroid hormone signaling pathway, etc. EGFR-TKI directly acts on the EGFR intracellular protein tyrosine kinase, which competitively binds to the tyrosine kinase with ATP, and inhibits tyrosine kinase phosphorylation, which in turn affects the processes of tumor cell proliferation, differentiation, invasion, and neovascularization, thus achieving the effect of targeted treatment of EGFR mutations[23] ; microRNAs in cancer can be abnormally expressed in various types of tumors and tumor tissues, and participate in tumor cell proliferation, migration, invasion, apoptosis, etc.[24] Thyroid hormone can promote angiogenesis, and anti-apoptosis, and inhibit the growth and metastasis of tumor cells.[25]

The molecular docking technique was used to investigate the binding between the key active ingredients and target proteins of Honeysuckle, and the results showed that quercetin and lignans had good binding activities with the targets EP000, ERBB2, GAPDH, MAPK3, etc., reflecting the fact that the main mechanism of the Honeysuckle's main active ingredients in the treatment of breast cancer may be closely related to the above components and the targets and that the Honeysuckle may exert certain therapeutic effects on breast cancer through multiple the Honeysuckle may have certain therapeutic effects on breast cancer through multi-targets and multi-signaling pathways.

In summary, this study applied the network pharmacology data platform to analyze and screen the main active ingredients of the honeysuckle and the disease targets of triple-negative

breast cancer, and meanwhile analyzed the multi-component, multi-target, multi-pathway mechanism of action of the honeysuckle in the treatment of triple-negative breast cancer, and the results showed that the honeysuckle may act on EP000, ERBB2, and GAPDH through a variety of active ingredients, such as the Quercetin and lignocellulosic acid, MAPK3 and other core targets, regulating triple-negative breast cancer through the cancer pathway, proteoglycan signaling pathway in cancer, microRNA pathway in cancer and thyroid hormone signaling pathway, and playing the roles of inhibiting tumor proliferation and metastasis, anti-inflammatory and regulating tumor cell microenvironment in the treatment of triple-negative breast cancer, which provides theoretical basis for the follow-up research and clinical treatment of triple-negative breast cancer. It provides a theoretical basis for subsequent research and clinical treatment of triple-negative breast cancer.

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