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Research Article

Uncovering the Mechanisms of Ranunculus Ternatus against Breast Cancer Based On Network Pharmacology and Molecular Docking

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Abstract

Breast Cancer (BC) is the primary cause of cancer death in women. Ranunculus Ternatus (RT) are widely used in China because of their anti-inflammatory activity and ability to anti cancer. But the mechanisms of anti breast tumor action are still unclear. In the present study we discovered its anti breast cancer mechanism based on network pharmacology and molecular docking. Public database were used to identify Breast Cancer gene and the target proteins of RT. Network topology and enrichment analyses were performed to screen for key targets and mechanisms. Finally, molecular docking tools were used to evaluate the drug and target binding. Seven compounds such as 7-O-Methyleriodictyol, Beta-sitosterol, Mandenol, Stigmasterol, CLR, and Truflex OBP and Sitosteryl acetate were identified as active ingredients. Enrichment analysis revealed that the underlying mechanisms were related to cell metabolic pathways , ovarian steroidogenesis, steroid hormone biosynthesis and chemical carcinogenesis - receptor activation. especially through ESR1, CYP19A1, HSD17B1, SHBG, CYP17A1, ESR2, AR and CYP1B1 signaling pathways. Molecular docking results revealed that all seven components of RT had good binding ability to their respective targets. In order to compare RT ingredients abilities, a known AR inhibitor drug, Apalutamide, was tested to dock to target AR. This study elucidated the potential molecular mechanism of RT in the treatment of BC, and it provides a basis for future experimental research and serves as a reference for new drug design.

Keywords: Breast Cancer; Molecular docking; Molecular mechanism; Network pharmacology; Ranunculus ternatus

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Introduction

Breast cancer (BC) is the second most common cancer and the leading cause of cancer mortality for women, accounting for 685,000 deaths worldwide in 2020 [1]. BC treatment includes: surgical resection, radioactive therapy, and target therapies. The most common side effects of treatment include fatigue, anemia, nausea/weight change/dietary issues etc. Development of effective and safe agents to reduce or reverse the incidence of breast cancer is necessary. Natural medical herbs is a kind of sources to be considered. Ranunculus Ternatus (RT) belongs to the buttercup family which consists of at least 62 genera and 2,200 species, and 42 genera and about 720 species are distributed throughout Mainland China [2]. Isolated phytometabolites of at least 17 genera have shown anti-cancer activities toward various tumor cells [3]. To date, traditional Chinese medicine containing RT has been reported to be effective against malignant lymphoma, leukemia, pulmonary tuberculosis, breast tumor, goiter, lung, thyroid, gastric, esophageal tumor etc [4-9]. But most of the anti-tumor effective constituents of ranunculus ternati and their mechanisms of action are still unclear. In the present study we focused on the potential active ingredients of Ranunculus ternatus and their anti breast cancer mechanism based on network pharmacology and molecular docking.

Network pharmacology is a new discipline based on systems biology theory, biological system network analysis, and multi target drug molecule design-specific signal node selection [10]. It has been widely used to study the molecular mechanisms underlying Chinese Medicine. Molecular docking is an established in silico structure-based method which can be used to model the interaction between a small molecule and a protein at the atomic level, which allows us to characterize the behavior of small molecules in the binding site of target proteins and elucidate fundamental biochemical processes [11]. Docking enables the identification of novel compounds of therapeutic interest, predicting ligand-target interactions at a molecular level, or delineating structure-activity relationships (SAR), without knowing a priori the chemical structure of other target modulators [12].

Therefore, network pharmacology and molecular docking was employed to explore the mechanism of action of RT in BC treatment.

Materials and Methods

Collection of the potential active compounds of RT

Potential pharmacologic active compounds of RT were collected from the TCMSP database [13], this study used OB≥30% and DL≥0.18 as the screening conditions. Total twelve compounds met the conditions. But five compounds did not have enough information such as 2D chemical structure or targets to do next step. Only seven active compounds were finally selected and used in the follow-up network pharmacological analysis.

Putative target prediction of RT

Putative targets of the active compounds were obtained from TCMSP, PubChem and Swiss Target Prediction databases. Cytoscape 3.9.1 software was used to visualize network diagrams of the active compounds and targets. Cytoscape is a software environment that is used for integrated models of biomolecular interaction networks.

Identification of BC related targets

Breast carcinoma and Breast cancer were used as keywords to identify relevant BC targets from the database. The following databases were used: OMIM, DrugBank, UniProt, GeneCards. we used the relevance score as a measure and selected targets (relevance score >4.1) based on the sample size or selected the top 20% of total targets if the sample size is too large.

Identification of overlapping targets of RT and BC

RT targets and BC targets were input into Excel 2016 to get overlapping targets. And both of them were input into Venn plot program of bioinformatics data base to get Venn diagram.

Protein-Protein Interactions (PPI) Network and RT Ingredient Target Network Construction

The overlapping target genes were input into the STRING database [14] to predict interactions between the genes by setting the organism to "Homo sapiens". Therefore, a PPI network graphic could be constructed. Then, Ingredient Target network map was established and visualized via Cytoscape software (version 3.9.1) to display the relationships between ingredients and targets.

Identification of core targets

The overlapping targets in the two databases of RT and BC were used as candidates for the mechanism of action of RT on BC. These related RT targets were then entered into the STRING tools software. The data analysis mode was set to "Multiple proteins," and the species was limited to "Homo sapiens." After screening the data, we set the confidence level to ≥0.40, hidden the isolated proteins, and exported a TSV file. Cytoscape 3.9.1 software was used to draw the PPI diagram. We also use "CytoNCA2.1.6" [15] to get core protein for the next step molecular docking use. The first 3 items that is, Betweeness (BC), Closeness (CC) and Degree (DC) were chosen to analyse setting as without weight.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

Overlapping targets were imported into the Functional Annotation tool of the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 [16]. The enriched P-values of the functional annotations were corrected using the Bonferroni ($P<0.05$) and Benjamini ($P<0.05$) methods [16]. The top 10 Gene Ontology molecular function (GOMF), biological process (GOBP) and cellular component (GOCC) and pathways with the highest enrichment were selected for analysis and display, GOBP, GOCC, GOMF and KEGG enrichment analyses were performed. A bubble chart, bar chart were plotted. The enrichment results were plotted using Bioinformatics, an online platform for data analysis and visualization.

Molecular docking

Macro molecular protein preparation: The official gene name of overlapping targets of RT and BC was up loaded to PDB data online to obtain the protein structure from the RCSB Protein Data Bank. PyMol2.5.0 modified the downloaded protein structure to remove original ligands and water molecules. The AutoDock Tools1.5.6 was used to add hydrogen and set the docking parameters, then output and saved as pdbqt type. The "Grid box" was set to perform the blind docking.

Ligand preparation: The ligand 3D structures were downloaded from the PubChem database in SDF format. Open Babel version 3.1.1 was used to mechanically convert to mol2 format. The AutoDock Tools1.5.6 was used to set the docking parameters, then output and saved as pdbqt type.

Molecular docking: AutoDock Vina1.1.2 was used to verify the ligand-protein binding affinity and the results of the pharmacological network. The PyMol2.5.0 software was used to visualize the binding results.

Results

RT active compounds and target network results

In the TCMSP database, subject word "Mao zhua Cao" as the search term, OB≥30% and DL≥0.18 as the screening conditions to obtain 7 active compounds. These identified compounds accounting for RT components with known active effects in table 1. Their molecule structures are in table 2. Thirty one hypothetical targets were identified associated with above 7 ingredients of RT.

Mol ID	Molecule Name	OB (%)	DL
MOL011319	Truflex OBP	43.74	0.24
MOL001494	Mandenol	42.00	0.19
MOL000242	7-O-Methyleriodictyol	56.56	0.27
MOL000358	Beta-sitosterol	36.91	0.75
MOL000449	Stigmasterol	43.83	0.76
MOL000953	CLR	37.87	0.68
MOL001973	Sitosteryl acetate	40.39	0.85

Table 1: Table of effective compounds of RT.

OB,oral bioavailability. DL, Drug likeness.

BC target network results

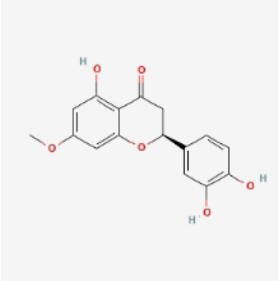
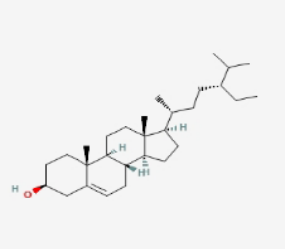
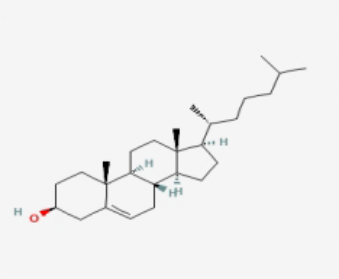
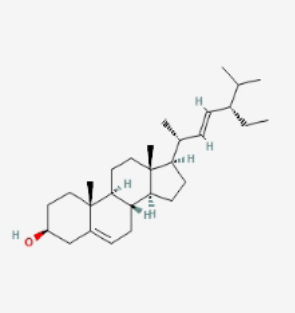
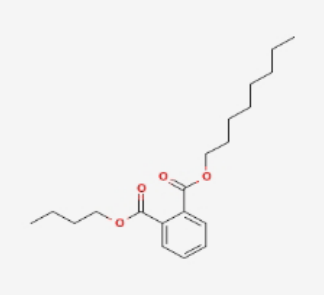
A total of 10181 genes were recorded as BC drug-disease related targets.

Overlapping targets of RT and BC

Based on the targets screened above, Bioinformatic SRplot online tools were used to map 31 drug-related targets with 10181 BC-related targets, resulting in 17 overlapping targets (as shown in figure 1).

Protein-Protein Interactions (PPI) Network and RT Ingredient Target Network

The 17 overlapping protein-protein interaction network was derived from STRING (as shown in figure 1). And RT Ingredient Target Network was derived from Cytoscape 3.9.1 (as shown in figure 2). Considering the information offered and the degree value from the compound-target network, top 7 compounds were chosen to be shown and to do further analysis, these are: Truflex OBP, beta-sitosterol, Mandenol, CLR, Stigmasterol, 7-O-methyleriodictyol and Sitosterol acetate.

No	Compound	Molecular Formula	Structure
1	7-O-Methyleriodictyol	C16H14O6	
2	Beta-sitosterol	C29H50O	
3	CLR	C27H46O	
4	Stigmasterol	C29H48O	
5	Truflex-OBP	C20H30O4	

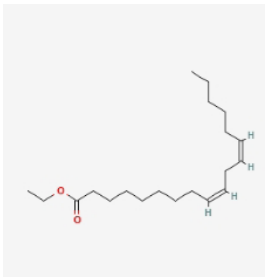
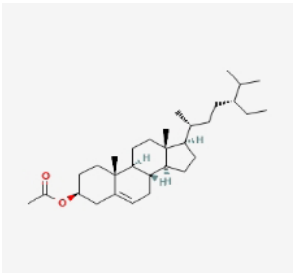
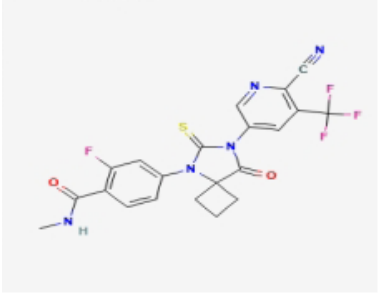
6	Mandenol	C20H36O2	
7	Sitosteryl acetate	C31H52O2	
8	Apalutamide (FDA approved drug)	C21H15F4N5O2S	

Table 2: Table of effective compounds of RT.

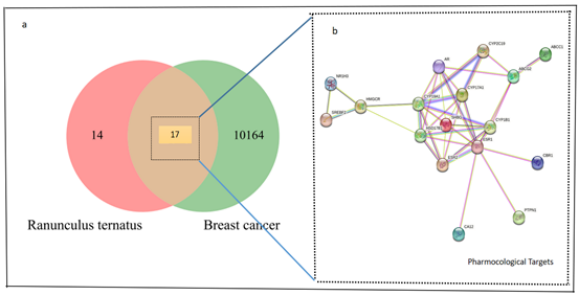


Figure 1: Venn diagram of intersecting targets of RT and BC and PPI network. (a) Venn diagram of intersecting targets of RT and BC. (b) PPI network of intersecting targets.

RT-ingredient-target -pathway-BC network

To further investigate the relationship between these ingredients, target genes, pathways and BC at the system level, the BC therapeutic targets, BC pathway and active ingredients of RT were mapped. Cytoscape 3.9.1 was used to construct a RT-ingredient-target -pathway-BC network (as shown figure 3). Nodes in different colors represent different groups: blue for targets, orange for ingredients, green for pathways, and purple for RT and BC. KEGG term name such as hsa04913 was Ovarian steroidogenesis etc (as shown in table 3).

Identification of core targets

The PPI protein interaction analysis was performed on 17 targets, the "cytoNCA" section was adopted to screen. As a result, these targets are illustrated (Figure 4) according to their score value.. The colors of the nodes are illustrated from red to yellow in descending order of degree values. The top 8 targets were chosen to do molecular docking, these were as followings: ESR1 (score=367), CYP19A1 (score=364), HSD17B1 (score=362), SHBG (score=360), CYP17A1 (score=242), ESR2 (score=240), AR (score=122) and CYP1B1 (score=122) (as shown in figure 4).

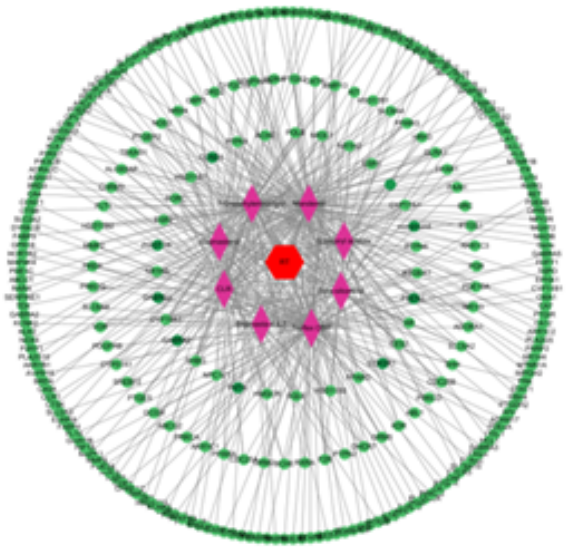


Figure 2: Compound-target network. Red hexagon represents RT, Purple diamond nodes represent active ingredients and green circular nodes represent corresponding targets.

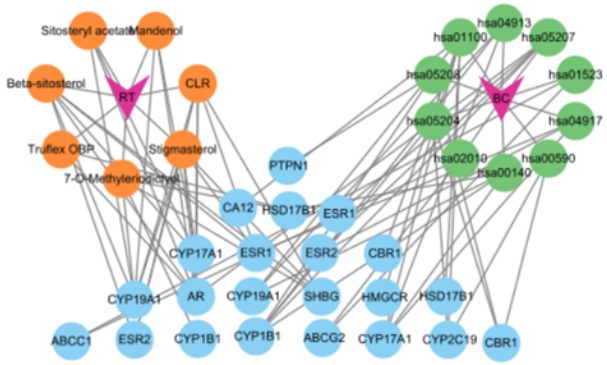
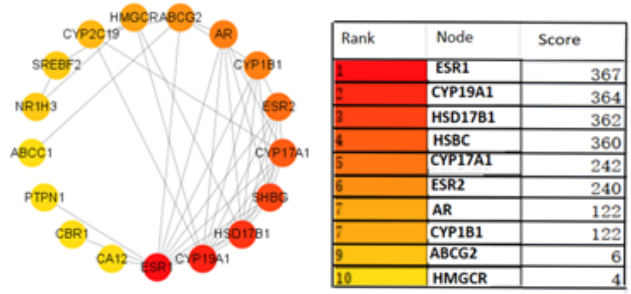


Figure 3: RT-ingredient-target-pathway-BC network. Nodes in different colors represent different groups: blue for targets, orange for ingredients, green for pathways, and purple for RT and BC.

Term
hsa04913:Ovarian steroidogenesis
hsa00140:Steroid hormone biosynthesis
hsa05207:Chemical carcinogenesis - receptor activation
hsa05204:Chemical carcinogenesis - DNA adducts
hsa04917:Prolactin signaling pathway
hsa01100:Metabolic pathways
hsa01523:Antifolate resistance
hsa05208:Chemical carcinogenesis - reactive oxygen species
hsa02010:ABC transporters
hsa00590:Arachidonic acid metabolism

Table 3: KEGG pathway term name.



Rank	Node	Score
1	ESR1	367
2	CYP19A1	364
3	HSD17B1	362
4	HSBC	360
5	CYP17A1	242
6	ESR2	240
7	AR	122
7	CYP1B1	122
9	ABCG2	6
10	HMGCR	4

Figure 4: The PPI protein interaction network and core targets of overlapping 17 targets. Top 10 targets were given degree score value. Data was collected from Cytoscape.

Enrichment analysis of GO and KEGG pathway

To further clarify the mechanism of RT treatment on BC, Seventeen targets were conducted an enrichment analysis with DAVID. Based on the gene counts and P- values, the top 10 KEGG pathways (Figure 5) and GO enrichment (Figure 6) were selected. For the KEGG pathway enrichment analysis, the targets were primarily enriched in Metabolic pathways (hsa01100), Ovarian steroidogenesis (hsa04913), Steroid hormone biosynthesis (hsa00140), Chemical carcinogenesis - receptor activation (hsa05207). For the biological processes, the targets were mainly enriched in intracellular steroid hormone receptor signaling pathway (GO:0030518), xenobiotic metabolic process (GO:0006805), intracellular estrogen receptor signaling pathway (GO:0030520), steroid biosynthetic and metabolic process (GO:0006694, GO:0008202). These results prove the primary mechanism of RT treatment of BC.

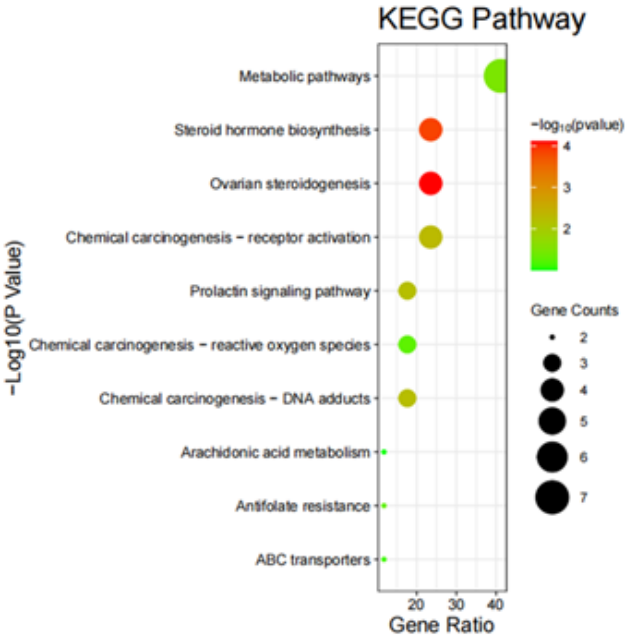


Figure 5: KEGG pathway analysis. The sizes of the bubbles are illustrated from big to small in descending order of the number of the potential targets involved in the pathways. Figure from bioinformatics.

Molecular docking analysis

Eight target genes (receptors) and their corresponding compounds (ligands) were selected for molecular docking analysis. During the

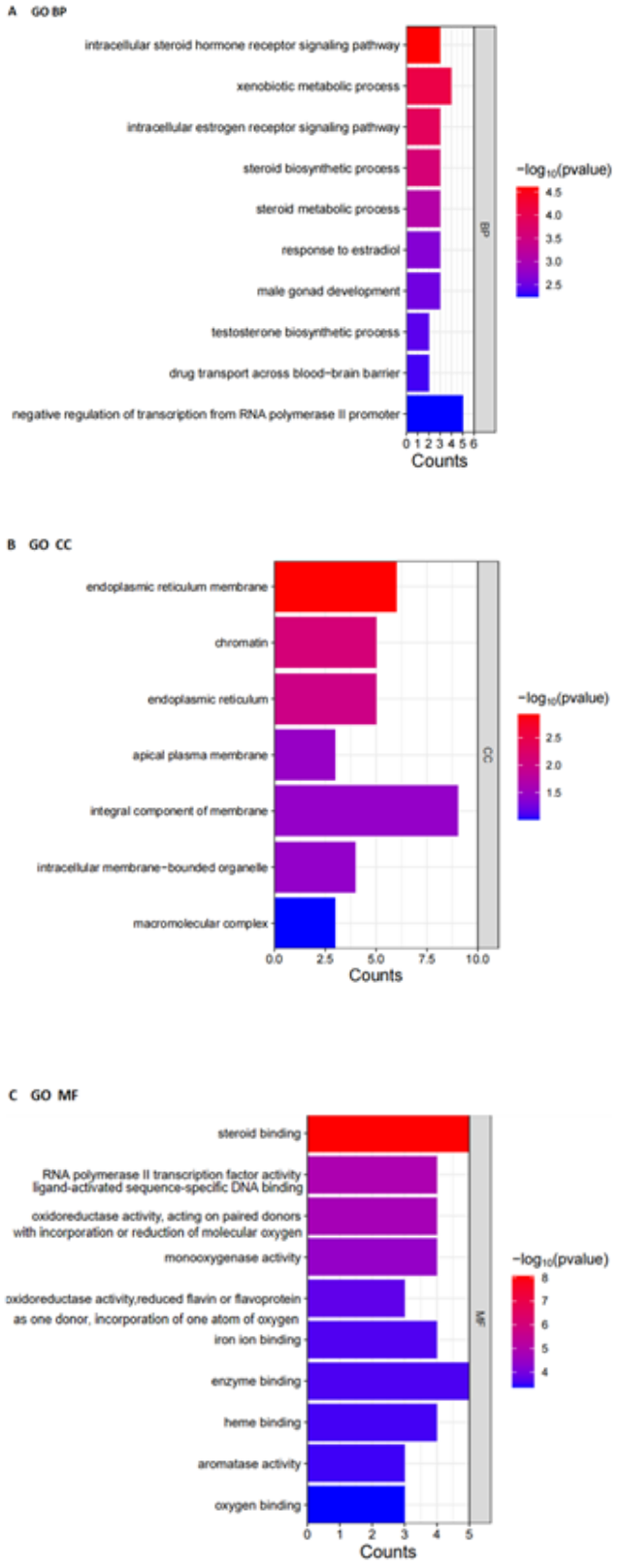


Figure 6: GO enrichment analysis. The top 10 terms of each part are shown. (A) BP: biological processes, (B) CC: cell component, (C) MF: molecular function. Figures were from bioinformatics.

docking process, binding free energy released due to the bond formation or interaction of protein-ligand. The Binding free energy at the active site can be used as an estimate of binding affinity. The lower the free energy the tighter the binding and the affinity. It is generally believed that binding energy <-4.25kcal/mol indicates certain binding activity between ligand small molecules and receptor proteins. The binding energy <-5.0kcal/mol indicated that there was a good binding activity between the two. Binding energy <-7.0kcal/mol indicates strong binding activity between ligand and receptor [17]. In addition to looking at binding energy, another evaluation index must be introduced. It was believed that the number of hydrogen bonds should also be included as an evaluation index [18]. We screened and displayed the 31 docking activity results (as shown in table 4). Figure 7 displays the docking patterns of the 31 complexes, including 8e1a with one approved drug apalutamide.

In figure 7, taking CYP19A1(3s79)→CLR as an example, the small molecule ligand CLR fits into the interfaced pocket formed by the CYP19A1(3s79) in the protein (Figure 7 (q) and (r)). The result showed that hydrogen bond formation was involved inLeu477(3.01Å), forthteen alkyl hydrophobic formations were involved in Glu302、Gly439、Phe148、Ile133、Arg115、Typ115、Trp224、Val370、Thr310、Leu152、Ala306、Met303、Phe203、Ala438. Therefore, CLR binds to CYP19A1 (3s79) through various interactions, mainly including hydrogen bonds and alkyl hydrophobicity. Further more, from table 4, except CYP17A1(6wr1) and Mandenol (-3.9kcal/mol), all the rest binding energy of the receptor-ligand complex <-5.0kcal/mol indicated that there was a good binding activity between them.

Discussion

In traditional Chinese medicine, RT exerts therapeutic effects on BC, but its mechanisms remain unclear. In the present study, the network pharmacology method was adopted to elucidate the relationship among active compounds, key targets and signaling pathways, thereby revealing the potential therapeutic mechanisms of RT. In this study, 7 active compounds, 8 key targets, and 4 pathways were ultimately predicted for the treatment of BC. The diversity of compounds, multiple key targets and pathways just embodies the principle of comprehensive treatment. Altogether, RT is a Chinese herbal with multi target therapeutic effects. Associations between these active compounds and BC warrant further investigation. This study identified ESR1, CYP19A1, HSD17B1, SHBG, CYP17A1, ESR2, AR and CYP1B1as the 8 hub protein targets related to BC.

Stigmasterol, CLR and Beta-sitosterol all had a very good binding ability to the core targets ESR1. ESR1 is a major ligand-activated transcription factor, member of the family nuclear receptors [19]. Estrogen receptor alpha (ERα), also known as NR3A1 is one of two main types of estrogen. In humans, ERα is encoded by the gene ESR1. The cellular action of estrogens is primarily mediated by the nuclear ERα, ERβ and the membrane G protein-coupled ER (GPER, called also GPR30). The ERα was considered to be the receptor most involved in the development of breast cancer [20]. It contributes to cancer progression as well as cancer inhibition, mutation of ERα is critical for cancer development and drug resistance, therefore constitutes a pivotal target for breast cancer therapy [21].

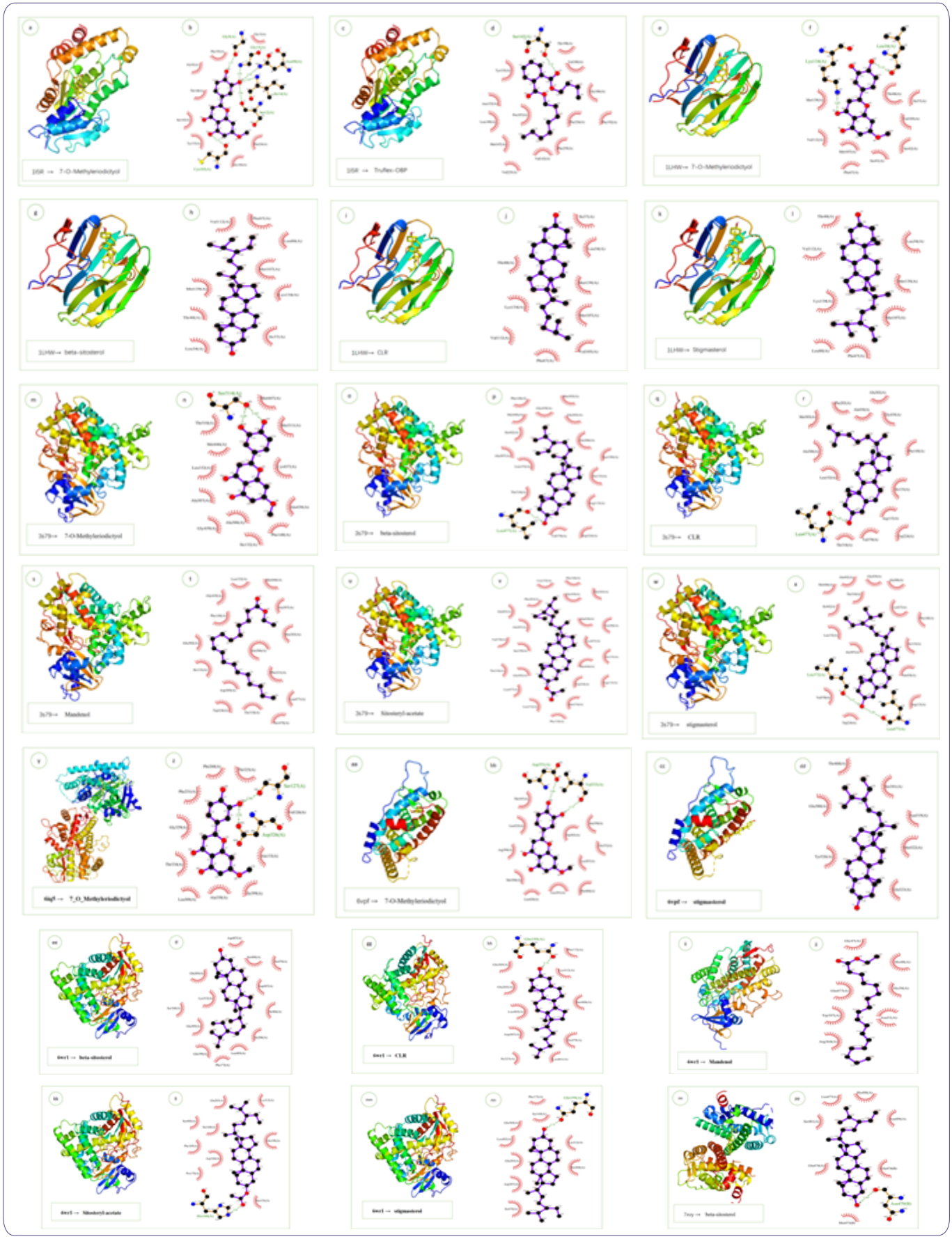
Mandenol, Beta-sitosterol, Stigmasterol, Truflex OBP, CLR and Sitosteryl acetate all had a very good binding ability to the core targets AR. The Androgen Receptor (AR), also known as NR3C4, is a type of nuclear receptor [22] that is activated by binding any of the

No.	Hub genes	PDB ID	Compound	Docking Affinity (kcal/mol)	Hydrogen bond position and length	Hydro-phobic action position
1	HSD17B1	1i5r (a)	O-Methyleriodictyol (b)	-8.8	Gly9(3.17Å)、Gly15(2.85Å)、Asn90(2.96Å)、Ile14(2.96Å)、Ser12(3.06Å)	Gly13、Phe226、Gly186、Tyr155、Ser142、Thr140、Gly92、Phe192
2	HSD17B1	1i5r (c)	Truflex-OBP (d)	-7.2	Ser142(3.31Å)、	Thr190、Val188、Gly186、Phe226、Phe192、Phe259、Val143、Val225、Met147、Leu149、Pro187、Asn152、Tyr155
3	SHBG	1lhw (e)	7-O-Methyleriodictyol (f)	-6.9	Leu34(3.14Å)、Lys134(3.05Å ;)	Thr40、Ile37、Val105、Ser42、Ser41、Phe67、Met107、Val112、Met139
4	HSBG	1lhw (g)	Beta-sitosterol (h)	-7.0	NO	Phe67、Leu80、Met107、Lys134、Ile37、Leu34、Thr40、Met139、Val112
5	HSBG	1lhw (i)	CLR (j)	-7.4	NO	Ile37、Leu34、Met139、Met197、Val105、Phe67、Val112、Lys134、Thr40
6	HSBG	1lhw (k)	Stigmasterol (l)	-8.0	NO	Leu34、Met139、Met107、Phe67、Leu80、Lys134、Val112、Thr40
7	CYP19A1	3s79 (m)	7-O-Methyleriodictyol (n)	-8.3	Ser314(3.03Å、3.05Å)	Met447、Met311、Cys437、Ala438、Phe148、Ile132、Ala306、Gly439、Ala307、Leu152、Met446、Thr310
8	CYP19A1	3s79 (o)	Beta-sitosterol (p)	-9.7	Leu477(3.07Å)	Met303、Glu302、Ala306、Ser199、Ile133、Arg115、Trp224、Val370、Thr310、Leu152、Ala307、Ile442、Met446、Phe148、Gly439
9	CYP19A1	3s79 (q)	CLR (r)	-10.0	Leu477(3.01Å)	Glu302、Gly439、Phe148、Ile133、Arg115、Typ115、Trp224、Val370、Thr310、Leu152、Ala306、Met303、Phe203、Ala438
10	CYP19A1	3s79 (s)	Mandenol (t)	-6.2	No	Met446、Ala307、Met303、Ala306、Phe221、Leu477、Ser478、Thr310、Trp224、Asp309、Ile133、Glu302、Phe148、Gly439、Leu152
11	CYP19A1	3s79 (u)	Sitosteryl-acetate (v)	-9.4	No	Phe148、Gly439、Met303、Ala438、Ala306、Cys437、Ile133、Met446、Trp224、Arg115、Met374、Phe374、Phe134、Leu372、Leu477、Thr310、Glu302、Val370、Ser199、Ala307、Ala443、Phe203、Leu152
12	CYP19A1	3s79 (w)	Stigmasterol (x)	-9.5	Leu477(3.80Å)、Leu372(3.55Å)	Gly439、Ala306、Cys437、Phe148、Ile133、Ala438、Arg115、Trp224、Val370、Ala307、Leu152、Ile442、Met446、Ala443、Thr310
13	CYP1B1	6iq5 (y)	7-O-Methyleriodictyol (z)	-8.9	Ser127(2.84Å)、Asp326(2.93Å)	Val126、Ala133、Ile399、Ala330、Leu509、Thr334、Gly329、Phe231、Phe268、Thr325
14	ESR1	6vpf (aa)	7-O-Methyleriodictyol (bb)	-8.5	Asp351(3.90Å)、Val533(3.75Å)	Ala350、Trp383、Glu353、Leu387、Phe404、Leu391、Leu428、Met388、Arg394、Leu525、Thr347

15	ESR1	6vpf (cc)	Stigmasterol (dd)	-6.8	NO	Glu523、Met522、Asn519、Ser381、Thr460、Glu380、Tyr526
16	CYP17A1	6wr1 (ee)	Beta-sitosterol (ff)	-6.7	NO	Phe172、Leu485、Ile206、Ile486、Asp207、Ile479、Ser488、Asp487、Glu203、Lys312、Ser168、Glu305、Gln199
17	CYP17A1	6wr1 (gg)	CLR (hh)	-7.0	Gln199(3.12Å)	Glu305、Glu203、Leu485、Asp207、Ile223、Lys481、Ile479、Ser488、Lys312、Phe172
18	CYP17A1	6wr1 (ii)	Mandenol (jj)	-3.9	No	Asn51、His50、His48、Gly47、Glu477、Trp397、Arg364
19	CYP17A1	6wr1 (kk)	Sitosteryl-acetate (ll)	-6.8	His160(3.04Å)	Met156、Gln199、Lys312、Glu203、Ser488、Ser168、Phe169、Asp166、Pro170
20	CYP17A1	6wr1 (mm)	Stigmasterol (nn)	-7.1	Glu199(3.18Å)	Lys312、Ser488、Ile479、Asp207、Glu203、Leu485、Glu305、Ser168、Phe172
21	ESR2	7xvy (oo)	Beta-sitosterol (pp)	-6.3	Asn470(3.69Å)	Met473、Glu474、Asn496、His498、Leu477、Ser481、Glu474
22	ESR2	7xvy (qq)	CLR (rr)	-6.1	Leu495(3.69Å)、Asn496(3.13Å)	Leu477、Glu474、His498、Met473、Leu477、Asn470、Met473
23	ESR2	7xvy (ss)	Stigmasterol (tt)	-6.7	Asn470(3.03Å)	Met473、Lys480、His498、Leu495、Asn496、、Ser481、Leu477、Glu474
24	AR	8e1a (uu)	Apalutamide (approved drug)(vv)	-8.4	Arg840(3.25Å)、Cys844(3.57Å)	Phe673、Leu674、Glu837、Ile672
25	AR	8e1a (ww)	Beta-sitosterol (xx)	-7.4	No	Leu674、Pro671、Phe673、Cys844、Glu837、Lys847、Arg846、Ala843、Arg840、Ile841
26	AR	8e1a (yy)	Stigmasterol (zz)	-7.7	Asp828(2.78Å)	Arg831、Phe916、Tyr857、Ile917、Ser853、Phe856、Ile835、Met832
27	AR	8e1a (a1)	Truflex-OBP (a2)	-7.2	Arg752(2.78Å)	Leu707、Gly708、Leu704、Met787、Met749、Met742、Leu873、Met745、Val715、Pro682、Lys808、Leu744、Trp718、Gln711、Ala748、Phe764
28	AR	8e1a (a3)	CLR (a4)	-7.4	No	Glu678、Asn675、Leu674、Gln802、Ile841、Arg846、Thr800、Lys845
29	AR	8e1a (a5)	Mandenol (a6)	-5.6	Trp751(3.07Å)	Leu805、Trp718、Val715、Leu744、Lys808、Pro682、Arg752、Gln711、Ala748、Glu681、Glu678、Pro801、Phe804
30	AR	8e1a (a7)	Sitosteryl-acetate (a8)	-7.3	Lys861(3.48Å)	Asp864、Pro868、Tyr915、Pro913、Lys912、Arg871、Thr918、His917、Ser865
31	ESR1	6vpf (a9)	Beta-sitosterol (b9)	-6.8	No	Phe425、Leu346、Leu525、Thr347、Met343、Asn532、Leu539、Leu354、Asp351、Val533、Ala350、Leu387、Trp383、Met388、Leu384

Table 4: Thirty one pairs docking result.

In the table, simble such as (a) refered to the figure number in fogure 7.



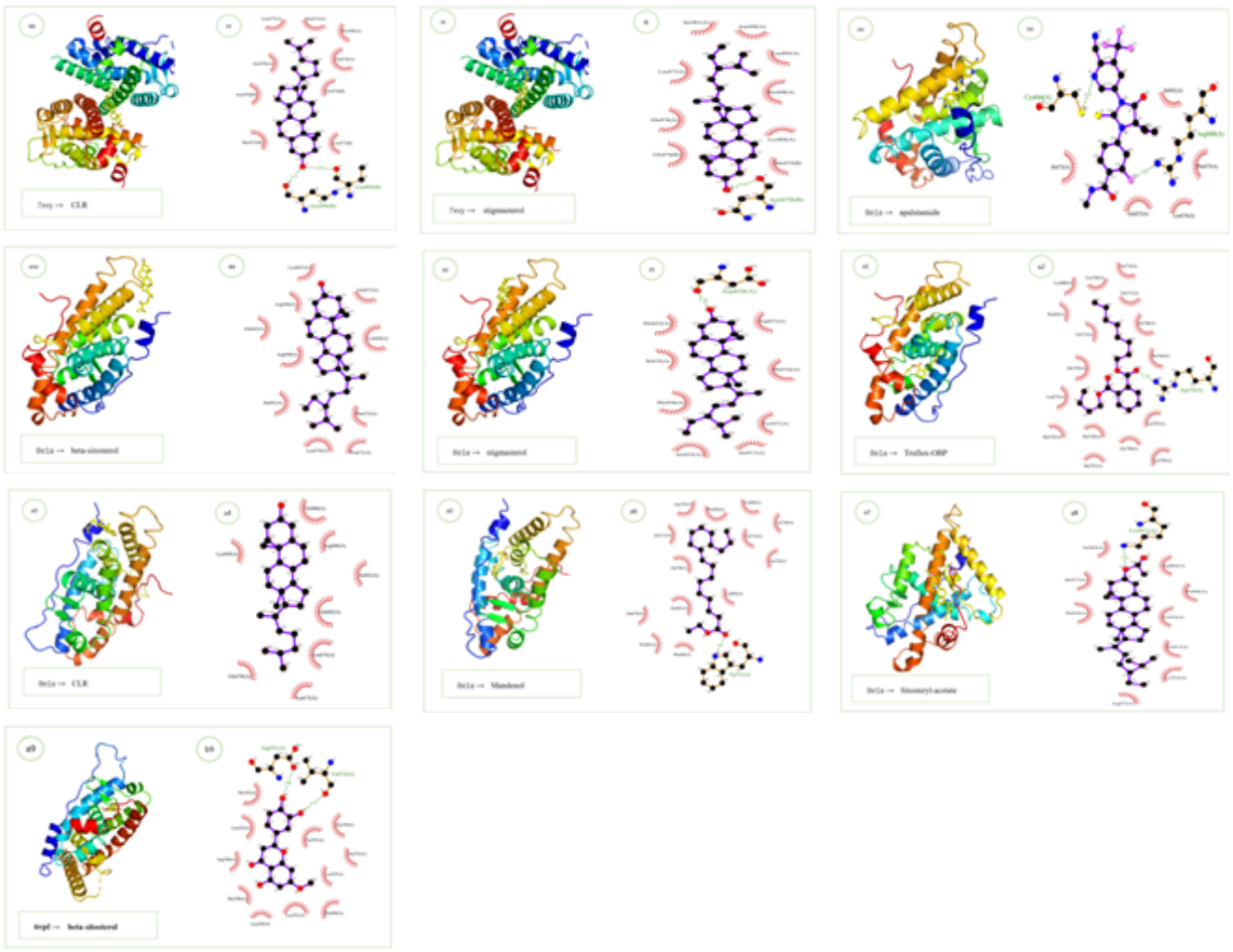


Figure 7: The docking patterns of the 31 complexes. Figures were from dock vina andPyMol2.5.0.

androgenic hormones, including testosterone and dihydrotestosterone [23] in the cytoplasm and then translocating into the nucleus. AR is expressed in 60%–70% of breast cancers, and may play a dual role with ER in both ER-positive cancers. ER−/AR+ breast tumors, exhibit an intact and active AR signaling [24,25]. Several pathways and mediators, including PI3K/mToR, HER2, BRCA1, cell cycle and immune modulation, can be tackled in LAR BCs and therefore have been exploited in AR- targeted therapies [26]. A known AR inhiibitor drug, Apalutamide, was tested to dock to terget AR. The binding energy is -8.4kcal/mol. While the RT ingredients such as Mandenol(-5.6kcal/mol), Beta-sitosterol(-7.4kcal/mol), Stigmasterol(-7.7kcal/mol), Truflex OBP(-7.2kcal/mol), CLR(-7.2kcal/mol) and Sitosteryl acetate(-7.3kcal/mol) demonstrated good ability to target AR comparing with Apalutamide. The latter ones may be potencial AR inhibitors which need to be studied in the future.

Mandenol, 7-O-Methyleriodictyol, Beta-sitosterol, Stigmasterol, CLR and Sitosteryl acetate all had a very good binding ability to the core target CYP19A1, which is a member of the cytochrome P450 superfamily catalyzing many reactions involved in steroidogenesis. CYP19A1 is responsible for the aromatization of androgens into estrogens. Elevated Aromatase (CYP19A1) Expression have been associated with increased risk and increased aggressiveness and Poor Survival of Patients with Estrogen Receptor Positive Breast Cancer [27,28]. Therefore CYP19A1 target therapy may be a new way for BC treatment.

Both Truflex OBP and 7-O-Methyleriodictyol had a very good binding ability to the core target HSD17B1, which oxidizes or reduces the C17 hydroxy/keto group of androgens and estrogens and hence is able to regulate the potency of these sex steroids. The implications for HSD17B1 as a treatment target have been known for a while, but the successful development of an inhibitor which can be brought into clinical trial is unfortunately not yet achieved [29].

Stigmasterol, 7-O-Methyleriodictyol, Beta-sitosterol and CLR all had a very good binding ability to the core target HSBG. Sex Hormone-Binding Globulin (SHBG) or Sex Steroid-Binding Globulin (SSBG) is a glycoprotein that binds to androgens and estrogens. High SHBG level is significantly associated with decreased risk of breast cancer in postmenopausal women, and it's a protective factor of breast cancer in postmenopausal women [30]. Less information can be found now for targeting SHBG for BC treatment and may be it is an interesting and new BC target therapy in the future.

Mandenol, Beta-sitosterol, Stigmasterol, CLR and Sitosteryl acetate all had a very good binding ability to the core target CYP17A1 which is an enzyme of the hydroxylase type that in humans is encoded by the CYP17A1 gene on chromosome 10. inhibition of CYP17A1 is a target for inhibiting the growth of hormone-dependent BC [31].

Beta-sitosterol, Stigmasterol and CLR all had a very good binding ability to the core target ESR2 which also known as NR3A2 is one of two main types of estrogen receptor—a nuclear receptor which is activated by the sex hormone estrogen. ESR2 expression is 2-fold higher in invasive BC as compared to normal breast tissues [32]. Same as ESR1, ESR2 genetic variants with altered risk of Triple-Negative Breast Cancer (TNBC) [33]. It shares structural homology at DNA and ligand binding domains (98% and 56%, respectively) with (ESR1) the major type of estrogen receptor in breast cancer [34,35]. And it is widely expressed in both basal and luminal epithelial cells [36-38]. The precise role of ESR2 in breast cancer is unclear, with both antiproliferative and proliferative roles described) [39,40].

O-Methyleriodictyol had a very good binding ability to the target CYP1B1. Human cytochrome P450 1B1 (CYP1B1) is an extrahepatic heme-containing monooxygenase. It plays an important role in the pathogenesis of hormone-related cancers and is responsible for anti-cancer drug resistance [41]. Cytochrome P4501B1 (CYP1B1) is elevated in breast cancer [42]. CYP1B1 enhances cell proliferation by inducing cell cycle transition and inhibiting cellular apoptosis in endometrial and breast cancer [43]. Inhibition of CYP1B1 activity is considered as an approach in cancer chemoprevention and cancer chemotherapy.

Conclusion

In summary, On the basis of network pharmacology and molecular docking, we predicted eight critical targets from the complex network analysis and provided a comprehensive explanation of the therapeutic mechanism of RT for BC, which may be related to proliferation, apoptosis, and immunity. In addition, ESR1, CYP19A1, HSD17B1, SHBG, CYP17A1, ESR2, AR and CYP1B1 signaling pathway may be critical for treating BC. Mandenol, 7-O-Methyleriodictyol, Beta-sitosterol, Stigmasterol, Truflex OBP, CLR and Sitosteryl acetate may be the main active ingredients of RT. Our research provides new ideas for the treatment of BC. However, further in vivo and in vitro experimental verification is expected to be conducted in the future.

Author's Contribution

Conceptualization, methodology, software, data curation, original draft preparation, Yolanda Sun; validation, formal analysis, writing review and editing, visualization and supervision, Chunguang Sun. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

- Our data acquisition method is described in detail and a detailed website link is listed below
- "TCMSP" at http://lsp.nwu.edu.cn/tcmsp.php
- "PubChem" at http://pubchem.ncbi.nlm.nih.gov;
- "Swiss Target Prediction" at http://www.swisstargetprediction.ch/
- "UniProt" at http://www.UniProt.org/;
- "DrugBank" at https://www.drugbank.ca/
- "GeneCards" at https://www.genecards.org/
- "OMIM" at http://omim.org/
- "bioinformatics" at https://www.bioinformatics.com.cn
- "STRING" at https://string-db.org/
- "DAVID" at https://david.ncifcrf.gov/
- "RCSB Protein Data Bank" at http://www.pdb.org/
- "AutoDockTools1.5.6"at http://mgltools.scripps.edu/documentation/links/autodock
- "Open Babel version 3.1.1" at http://openbabel.org

Conflicts of Interest

The authors declare no conflict of interest.

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