Research Article

Probing the mechanism of action (MOA) of *Solanum nigrum* Linn against breast cancer using network pharmacology and molecular docking

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Abstract
Solanum nigrum Linn is a medicinal herb widely used in traditional Chinese medicine to treat ailments such as fever, inflammation and cancer. Although quite a few compounds have been isolated and characterized, its anticancer mechanism remains elusive. Thus, in this study, we used network pharmacology and molecular docking strategies to identify the major active ingredients in *S. nigrum* and reveal its putative mechanism against human breast cancer. Six compounds, quercetin, cholesterol, 3-epi-beta-sitosterol, diosgenin, medioresinol and solanocapsine, were identified to be the major active ingredients. Target identification and analysis showed that they regulate 80 breast cancer-related targets. Furthermore, network analysis showed that the six active ingredients regulate multiple pathways including ErbB signaling pathway and estrogen signaling pathway and genes *AKT1*(AKT serine/threonine kinase 1), *ESR1*(estrogen receptor 1), *EGFR*(epidermal growth factor receptor), *SRC*(proto-oncogene tyrosine-protein kinase Src), *AR*(androgen receptor) and *MMP9*(matrix metalloproteinase 9) are crucial genes involved in the regulations. Molecular docking implied that quercetin could form good binding with AKT1, EGFR, SRC and MMP9. Our current study suggests that the anticancer function of *S. nigrum* is likely via synergistic/additive effects of multiple active ingredients' regulations of different signaling pathways. Further studies are warranted to establish the standard for *S. nuigrum* to be used as a CAM (complementary and alternative medicine) in breast cancer treatment and dissect its potential interactions with chemotherapy drugs.

Keywords *Solanum nigrum* L. · Breast cancer · Mechanism of action · Network pharmacology · Molecular docking

Abbreviations

S. nigrum	*Solanum nigrum* Linn
CAM	Complementary and alternative medicine
TCM	Traditional Chinese medicine
MOA	Mechanism of action
TCMSP	Traditional Chinese medicine systems pharmacology database and analysis platform
OB	Oral bioavailability
DL	Drug-likeness
PPI	Protein–protein interaction
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BP	Biological process
CC	Cellular component
MF	Molecular function
AKT1	AKT serine/threonine kinase 1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
SRC	Proto-oncogene tyrosine-protein kinase Src
MMP9	Matrix metalloproteinase 9

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ESR1	Estrogen receptor 1
AR	Androgen receptor
TNBC	Triple-negative breast cancer
ECM	Extracellular matrix
TCGA	The Cancer Genome Atlas

1 Introduction

Breast cancer is one of the leading causes of cancer death in women. The latest epidemiological report shows that female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer with 2.3 million new cases (11.7% of total cases) worldwide in 2020, closely followed by lung cancer (11.4%) and colorectal cancer (10.0%) [1]. The incidence rate of breast cancer has been decreased slightly in Canada over the past 30 years [2] but increased at 0.5% per year in the United States in the past years [3]. In addition, it has been noticed that the affected population is getting younger [4], which is highly concerning in the society. However, the mortality rate of breast cancer has been decreased steadily in both Canada and the United States since 1990s [3, 5], which is mainly attributed to advances in breast cancer diagnosis and treatment, such as mammography, targeted therapy and immunotherapy.

Although the 5-year survival rate for breast cancer is around 90%, prognosis for advanced stage and recurrent breast cancer remains poor [6, 7]. For example, the 5-year survival rate is around 100% for stage I patients but 22% for stage IV patients in Canada [6]. Chemotherapy of breast cancer usually faces drug resistance and severe adverse drug reactions, which, in turn, significantly affects the quality of life in breast cancer patients [8, 9]. Furthermore, the high cost of oncology drugs may limit their clinical application in certain countries [10]. Thus, many cancer patients, not just limited to advanced stage patients, seek complementary and alternative medicine (CAM) treatments in an attempt to improve therapeutic efficacy and/or reduce adverse drug reactions. A study of cancer patients in Northern Ontario, Canada showed that 51.8% of the patients used CAM products after diagnosis [11]. For patients' safety, it is critical to enhance researches to understand the mechanism of action (MOA) and side effects of the CAM products.

Medicinal plants are a valuable resource for developing anticancer therapeutics and widely used in CAM. For example, paclitaxel, a chemotherapy drug used to treat breast cancer and ovarian cancer, was discovered from the Pacific Yew tree. *Solanum nigrum* L., commonly known as black nightshade, is a folklore herb used in traditional Chinese medicine (TCM). It is usually used to treat ailments such as fever, pain and inflammation [12–14]. However, its anticancer function has attracted people's attention in recent years [15–18]. The water extract of *S. nigrum* and various active ingredients such as polyphenols have shown potent *in vitro* anticancer activities [19–22]. Specifically for breast cancer, it has been reported that *S. nigrum* can induce apoptosis and autophagy [15] and suppress mitochondrial function and epithelial-mesenchymal transition [19] in breast cancer cells. In addition, our previous studies showed that *S. nigrum* water extract inhibited cell migration and suppressed aerobic glycolysis towards human breast cancer MCF7 cells [23].

The main active components of *S. nigrum* and their corresponding targets in human cells have not yet been thoroughly identified, though. The underlying MOA for the entire plant rather than just an active constituent should be clarified because *S. nigrum* is typically administered as a whole plant in TCM preparations. In the present study, active components of *S. nigrum* as well as their potential mechanism behind its anticancer action were investigated by using network pharmacology and molecular docking approaches.The findings of this investigation may be able to offer a fresh method for thoroughly comprehending its antineoplastic properties..

2 Materials and methods

2.1 Screening of active ingredients in S. nigrum and corresponding targets in human cells

Information of bioactive compounds in *S. nigrum* were retrieved from the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) (https://tcmspw.com/tcmsp.php) using "*Solanum nigrum* Linn" as a keyword. Oral bioavailability (OB ≥ 30%) and drug-likeness (DL ≥ 0.18) were set as the thresholds in identifying candidate active ingredients [24, 25]. For each candidate, 3D structure and structural graphic were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and saved in sdf and mol2 format. Subsequently, the sdf file was uploaded to Swiss Target Prediction database (http://swisstargetprediction.ch/index.php) to identify its potential interacting protein targets. Target protein names filtered with "Homo sapiens" and "Probability > 0" were downloaded.

2.2 Prediction of breast cancer-related targets for S. nigrum Active Ingredients

All breast cancer-related target genes were retrieved from GeneCards database (https://www.genecards.org/) using

"breast cancer" as a keyword. These targets were subsequently standardized in UniProt (http://www.uniprot.org/) with organism selected as "Homo sapiens" to obtain a list of human breast cancer related targets. Finally, common targets between human breast cancer-related targets and the predicted targets for *S. nigrum* active ingredients (Sect. 2.1) were extracted and presented in a Venn diagram.

2.3 Construction of protein–protein interaction (PPI) network

The aforementioned common targets (Sect. 2.2) were subjected to protein-protein interaction networks and functional enrichment analysis using STRINGdb (https://string-db.org/). The protein interaction data was then imported into Cytoscape 3.7.2 [26] to construct PPI, analyze key regulatory proteins in the PPI, and identify core proteins with maximal degrees in topological analysis.

2.4 GO and KEGG analysis of key protein targets

Key targets identified above in Sect. 2.2 were uploaded to DAVID (database for annotation, visualization and integrated discovery, https://david.ncifcrf.gov/) for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (http://www.genome.jp/kegg/) with the species defined as "Homo Sapiens" and the threshold of significant difference set at $p < 0.05$. GO function enrichment analysis includes biological process (BP), cellular component (CC) and molecular function (MF). The results were then visualized and plotted using an online bioinformatics tool for data analysis and visualization (http://www.bioinformatics.com.cn).

2.5 Construction of component-target-pathway network

To figure out MOA of *S. nigrum* against breast cancer, we constructed the component-target-pathway network using the aforementioned information of active ingredients (component), key protein targets (target) and major biological pathways (pathway). The network was subsequently visualized using Cytoscape 3.7.2. In the network, the nodes represent components, targets and associated pathways (shown in pink, blue and green, respectively); while the edges represent component-target and target-pathway interactions.

2.6 Molecular docking of major active ingredients to key protein targets

Molecular docking was used to evaluate the capability and affinity of *S. nigrum* active ingredients to the selected key protein targets. 3D-structures of the protein targets were retrieved from the RCSB Protein Data Bank (PDB, http://www1.rcsb.org/) and 3D-structures of the active ingredients were prepared using Chimera 1.15 [27]. Molecular docking was performed using AutoDock Vina [28], with binding energy of −5.0 kcal/mol as the selection criteria [29].

3 Results

3.1 Identification of S. nigrum active ingredients and their respective targets in human cells

Seven major active ingredients (OB ≥ 30% and DL ≥ 0.18) were retrieved for *S. nigrum* from the TCMSP database. They are cholesterol, solanocapsine, diosgenin, medioresinol, beta-carotene, quercetin and 3-epi-beta-sitosterol (Table 1). Then, 190 potential targets were identified for the 7 bioactive compounds from the Swiss Target Prediction database.

3.2 Identification of bioactive compounds targets in S. nigrum for breast cancer

We extracted 14,360 breast cancer-related gene targets from GeneCards and selected 1837 genes for further crossover analysis after two cycles of median operation (score ≥ 9.67). As shown in Fig. 1, 80 gene targets were revealed to be shared between the bioactive compound targets and breast cancer targets. They are related to 6 active ingredients of *S. nigrum* except beta-carotene. Thus, beta-carotene was removed from the active ingredient list in the following analyses.

Table 1 Major active ingredients identified in *S. nigrum*

No	Active ingredient	PubChem CID	OB (%)	DL
S1	Cholesterol	5997	37.87	0.68
S2	Solanocapsine	73,419	52.94	0.67
S3	Diosgenin	99,474	80.88	0.81
S4	Medioresinol	181,681	57.2	0.62
S5	Beta-carotene	5,280,489	37.18	0.58
S6	Quercetin	5,280,343	46.43	0.28
S7	3-Epi-beta-sitosterol	12,303,645	36.91	0.75

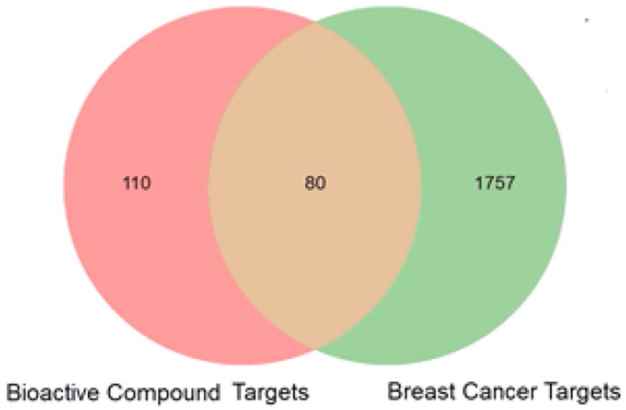

Fig. 1 Venn diagram showing the *S. nigrum* bioactive compound targets and breast cancer targets

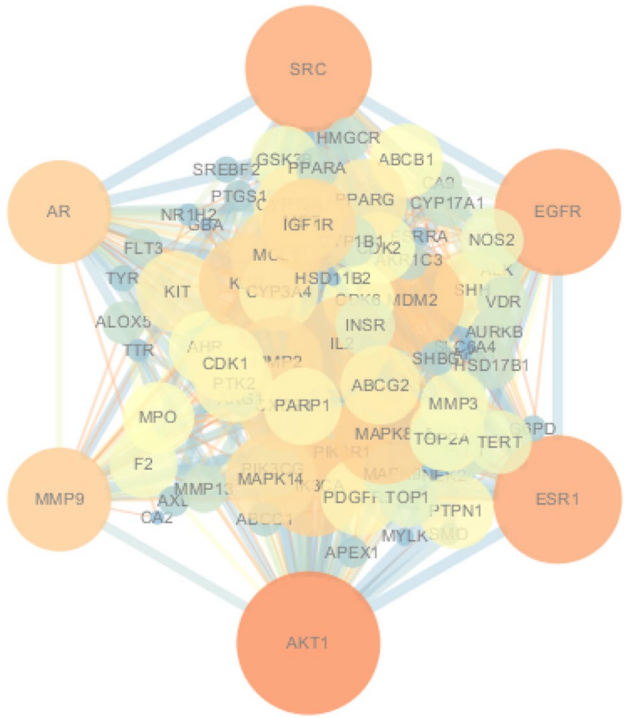

Fig. 2 PPI network for the 80 common targets between *S. nigrum* bioactive compound targets and breast cancer targets

3.3 PPI Network Construction

PPI network was constructed for the 80 identified targets using the STRING database with the minimum interaction score set at 0.4 (Fig. 2). After screening, 79 targets were identified to interact with other proteins and 634 edges were observed in the network. This indicates that multiple interactions are present for the targets. The average degree of each node was 15.8. Sucrase-isomaltase (encoded by gene *SI*) was removed from the PPI network as it did not meet the selection criteria. Furthermore, we noticed that six proteins with highest degrees in the PPI network could be major hubs responsible for the anticancer function of *S. nigrum*. The six hub proteins are AKT1 (AKT serine/threonine kinase 1), ESR1 (estrogen receptor 1), EGFR (epidermal growth factor receptor), SRC (proto-oncogene tyrosine-protein kinase Src), AR (androgen receptor) and MMP9 (matrix metalloproteinase 9).

3.4 GO and KEGG enrichment analysis

DAVID database was employed for GO enrichment analysis to reveal the biological functions associated with the 80 common targets ($p < 0.05$). In total, we obtained 211 enrichment items for BP, 27 enrichment items for CC and 71 enrichment items for MF. In Fig. 3, we presented the top 10 enriched terms in each GO category in the order of *p*-value from low to high. These top 10 enriched terms are protein autophosphorylation, negative regulation of apoptotic process, peptidyl-tyrosine phosphorylation, response to drug, positive regulation of cell proliferation, positive regulation of gene expression, positive regulation of transcription from RNA polymerase II receptor, transmembrane receptor protein tyrosine kinase signaling pathway, oxidation–reduction process and phosphatidylinositol-mediated signaling in BP; cytosol, nucleus, nucleoplasm, plasma membrane, protein complex, extracellular space, extracellular exosome, mitochondrion, phosphatidylinositol 3-kinase complex and cytoplasm in CC; and ATP binding, transmembrane receptor protein tyrosine kinase activity, RNA polymerase II transcription factor activity, enzyme binding, protein tyrosine kinase activity, protein kinase activity, kinase activity, steroid binding, steroid hormone receptor activity and protein binding in MF.

Signaling pathway enrichment for the 80 common targets was performed using the KEGG pathway database in order to reveal the key signaling pathways in breast cancer that are regulated by the active ingredients of *S. nigrum*. We identified 83 primarily enriched signaling pathways. The top 20 most significantly enriched pathways ($p < 0.05$) are pathways in cancer, prolactin signaling pathway, proteoglycans in cancer, FoxO signaling pathway, focal adhesion, central carbon metabolism in cancer, progesterone-mediated oocyte maturation, prostate cancer, PI3K-AKT signaling pathway, melanoma, insulin resistance, ErbB signaling pathway, glioma, ovarian steroidogenesis, estrogen signaling pathway, Rap1 signaling pathway, Ras signaling pathway, VEGF signaling pathway, pancreatic cancer and hepatitis C (Fig. 4).

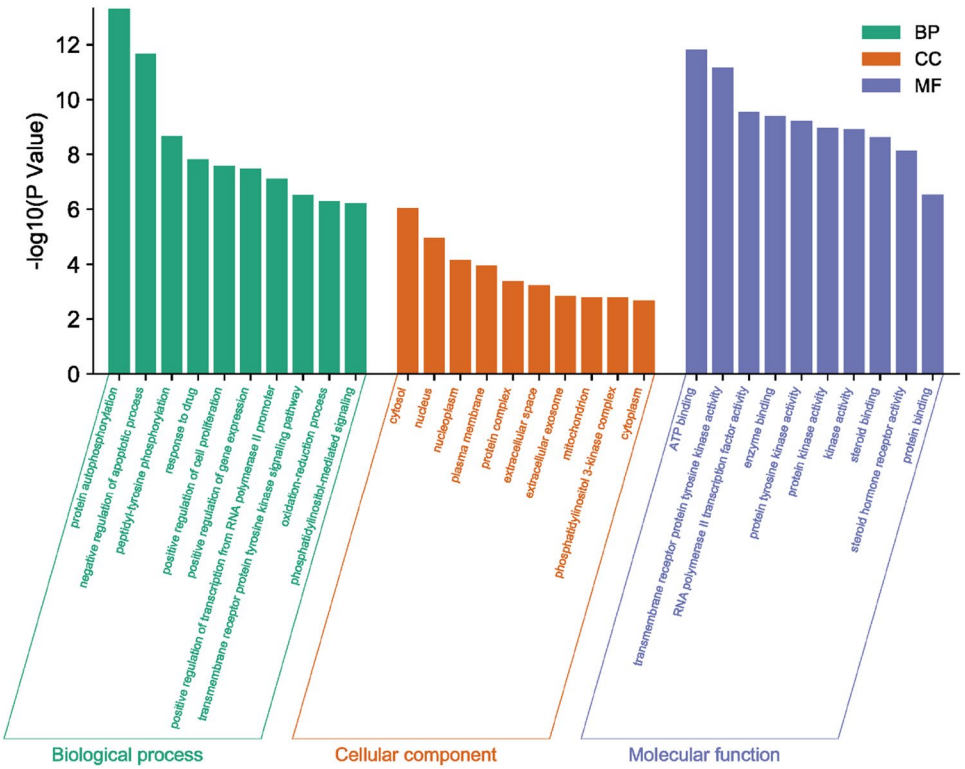

Fig. 3 Top 10 enriched items (p-value from low to high) for the 80 common targets (identified in Sect. 3.2) in the three GO categories of biological process (BP, shown in green), cellular component (CC, shown in orange) and molecular function (MF, shown in blue)

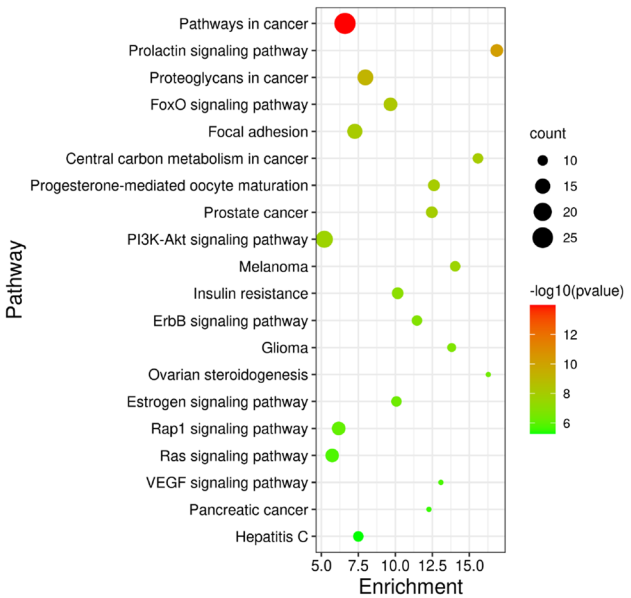

Fig. 4 Top 20 most significantly enriched signaling pathways regulated by the active ingredients of *S. nigrum* in breast cancer (revealed by KEGG pathway analysis). Dot color and size represent p-value range and gene number mapped to the indicated pathways, respectively

3.5 Construction of component-target-pathway network

The component-target-pathway network (Fig. 5) was constructed using Cytoscape 3.7.2 by incorporating the 6 bioactive ingredients of *S. nigrum*, the 80 common targets shared between the bioactive compound targets and breast cancer targets, and the top 20 signaling pathways revealed by KEGG analysis. The network degree value was calculated for the 6 bioactive ingredients (Table 2) with 4 compounds having the value higher than 10. The 4 compounds are quercetin, cholesterol, 3-epi-beta-sitosterol and diosgenin. The network degree was relatively low for medioresinol and solanocapsine. This network analysis suggests that quercetin may play a more important role in contributing to the anticancer function of *S. nigrum* than the other active ingredients. It is also noteworthy that each active ingredient acts on multiple targets and is involved in various signaling pathways, implying that synergistic, additive and even antagonistic effects might be present among the active ingredients. Further studies are warranted to confirm these effects.

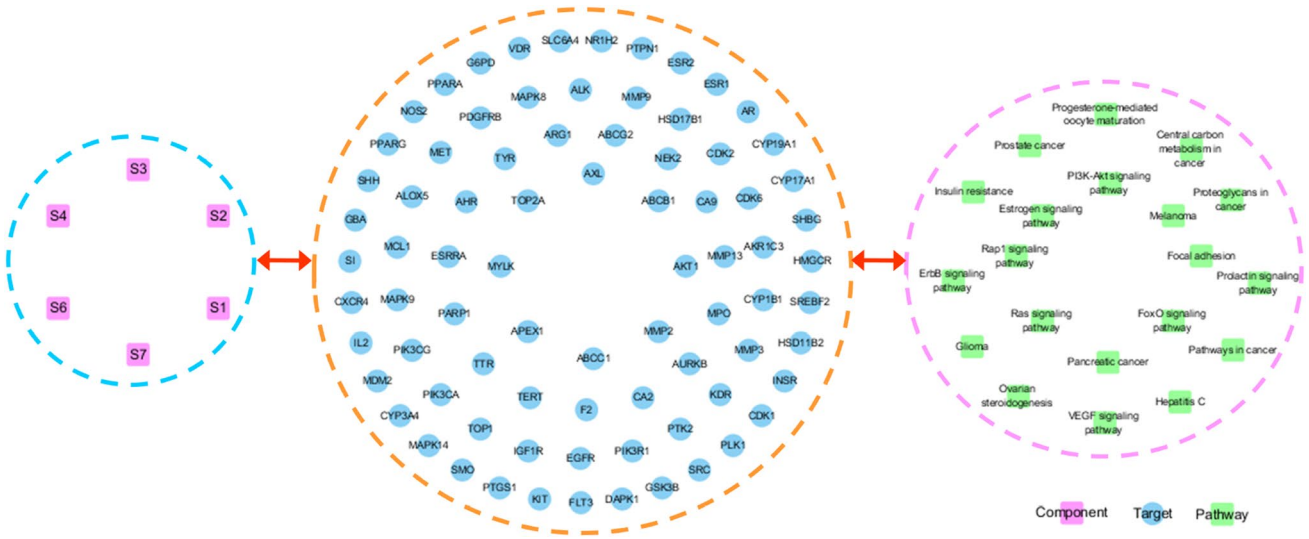

Fig. 5 The component-target-pathway network constructed for *S. nigrum*. The component (active ingredients of *S. nigrum*), target (80 common targets identified in Sect. 3.2) and pathway (top 20 signaling pathways identified from KEGG) were shown in pink, blue and green, respectively

Table 2 Network degree values of the 6 active ingredients of *S. nigrum*

Component No	Chemical compound	Degree
S6	Quercetin	49
S1	Cholesterol	17
S7	3-Epi-beta-sitosterol	17
S3	Diosgenin	14
S4	Medioresinol	7
S2	Solanocapsine	3

3.6 Docking the active ingredients of S. nigrum to the six hub proteins revealed from PPI

From the above PPI analysis, we identified 6 key protein targets (AKT1, ESR1, EGFR, SRC, AR and MMP9), which could be the major biological hubs responsible for the anti-breast-cancer function of *S. nigrum*. To explore whether the active ingredients could bind directly to these hub proteins, we carried out the docking studies between the 6 bioactive compounds and the 6 hub targets using binding energy of -5.0 kcal/mol as a selection cutoff. A binding energy value of less than 0 suggests indicates that the ligand molecules can spontaneously bind to the receptor protein.The lower the binding energy value,the stronger the affinity between the two. A value of less than – 5.0 kal/mol suggests that the ligand molecules have a desirable binding affinity.The results of the molecular docking revealed that 8 ingredient-target pairs were lower than –5 kcal/mol, indicating that 3 active ingredients, namely quercetin, cholesterol and 3-epi-beta-sitosterol, had good binding affinity to each of the six hub protein targets, namely AKT1,ESR1,EGFR,SRC,AR and MMP9 (Table 3). The docking patterns of the core ingredients and hub proteins were shown in Fig. 6. This docking

Table 3 Binding energy of *S. nigrum* active ingredients to the key protein targets

Hub protein target	Active ingredient	Binding energy (kcal/mol)	Referred binding energy (kcal/mol)
AKT1	Quercetin	–6.7	–9.7 to –5.36[30–34]
ESR1	Cholesterol	–6.7	–6.5[35]
ESR1	3-Epi-beta-sitosterol	–6.6	–10.09 to –6[35–41]
EGFR	Quercetin	–7.5	–8.8 to –5.25[31, 42–46]
SRC	Quercetin	–9.7	–10.543 to –7.9[31, 45, 47–49]
AR	Cholesterol	–6.8	–7.6[35]
AR	3-Epi-beta-sitosterol	–7.1	–9.56 to –6.8[35, 39, 50]
MMP9	Quercetin	–9.4	–9.7 to –5.15[35, 44, 48, 51, 52]

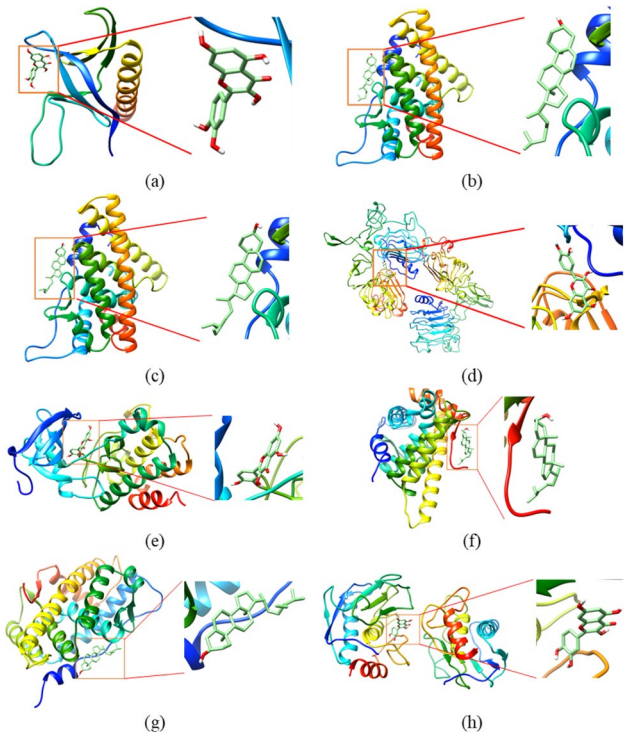

Fig. 6 Conformations of the 8 strong bindings identified from molecular docking between the 6 active ingredients of *S. nigrum* and the 6 hub protein targets, **a** AKT1 and quercetin, **b** ESR1 and cholesterol, **c** ESR1 and 3-epi-beta-sitosterol, **d** EGFR and quercetin, **e** SRC and quercetin, **f** AR and cholesterol, **g** AR and 3-epi-beta-sitosterol, and **h** MMP9 and quercetin

study shows that quercetin could potentially bind to four hub targets, including AKT1, EGFR, SRC, and MMP9, which may trigger synergistic effects among different signaling pathways, suggests the use of quercetin in the treatment of breast cancer and provides data support for further experimental design.

4 Discussion

S. nigrum is a medicinal herb widely used to treat fever, pain and inflammation in TCM [12–14]. With the discovery of its anticancer activities [15–18], *S. nigrum* has been included in integrative therapy of TCM to treat different types of cancer. Our previous studies have shown that the water extract of *S. nigrum* inhibited proliferation and migration of breast cancer MCF7 cells and suppressed the cells' aerobic glycolysis [23]. However, the anticancer mechanism of *S. nigrum* is still far from understanding.

In the present study, we undertook an approach including network pharmacology and molecular docking to probe MOA of *S. nigrum* against human breast cancer. Six major bioactive compounds, cholesterol, solanocapsine, diosgenin, medioresinol, quercetin and 3-epi-beta-sitosterol, were identified. Out of these bioactive compounds, quercetin, which is probably one of the most-studied nutraceuticals, has been shown to possess strong antioxidant and anti-inflammatory activities and induce apoptosis of breast cancer cells [53, 54]. It can also inhibit cell adhesion and migration of breast cancer cells via downregulating HuR protein [54]. β-sitosterol, which is the most abundant type of phytosterol in various dietary plants such as vegetables and nuts, can inhibit the growth of breast cancer cells [55, 56]. Diosgenin, a steroidal saponin in plants, is also capable of inhibiting the growth and migration of human breast cancer cells [57, 58]. However, the functions of cholesterol, solanocapsine and medioresinol towards breast cancer cells are unknown.

Our subsequent analyses showed that the 6 active ingredients of *S. nigrum* interact with 80 breast-cancer-related targets and regulate a wide range of biological pathways. From the PPI network, 6 key protein targets (AKT1, ESR1, EGFR, SRC, AR and MMP9) were identified as major biological hubs. They play important roles in normal breast functions, and upregulation of their expressions promote breast cancer proliferation and metastasis. Therefore, we examined the expressions of the genes encoding these 6 hub proteins in breast cancer patients using online software GEPIA (http://gepia.cancer-pku.cn/). As shown in Fig. 7, genes *ESR1*, *AR* and *MMP9* are upregulated and gene *EGFR* is downregulated, respectively, in breast cancer patients ($p < 0.05$ and labeled with *). However, none of them is a prognostic marker for breast cancer.

For the four differentially expressed genes in breast cancer patients, gene *ESR1* encodes ESR1, which is a major hormone receptor on the surface of breast cancer cells. Its upregulation stimulates breast cancer proliferation, growth, and metastasis [59, 60]. *ESR1* introns SNP + 2464 C/T (rs3020314) and SNP -4576 A/C (rs1514348) are correlated with breast cancer susceptibility and the expression status of progesterone receptor [61]. EGFR enhances proliferation and invasion of tumor cells and promotes breast cancer progression via JAK/STAT3 signaling [62]. EGFR is a major target for ΔNp63 regulation that influences cancer cell adhesion in basal-like triple-negative breast cancer (TNBC) [63]. Although *EGFR* is downregulated in breast cancer patients (without subcategorization), it is overexpressed in up to 76% in TNBC patients and EGFR-based targeted therapy has shown promising effects in TNBC patients [64, 65]. AR is expressed in > 70% breast cancer and across three major breast cancer subtypes [66, 67]. However, it seems that the function of AR is subtype-specific [67]. AR signaling can also crosstalk with other signaling pathways to regulate breast cancer pathology and progression [67]. It can either elicit or diminish oncogenic effects in breast cancer depending

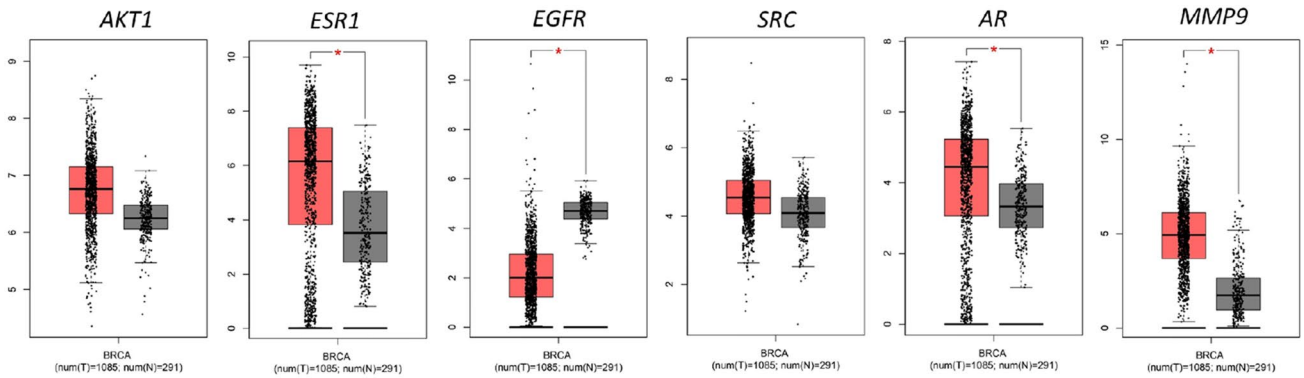

Fig. 7 Boxplots showing the expressions of genes AKT1, ESR1, EGFR, SRC, AR and MMP9 in breast cancer patients (shown in red) versus normal controls (shown in grey) in the patient dataset BRCA deposited in The Cancer Genome Atlas (TCGA). This figure was generated using online software GEPIA (http://gepia.cancer-pku.cn/)

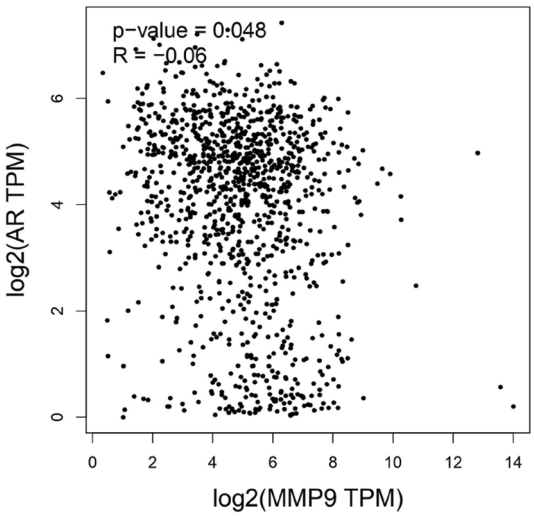

Fig. 8 Pearson correlation between the expressions of MMP9 and AR in the patient dataset BRCA deposited in TCGA. This figure was generated using online software GEPIA (http://gepia.cancer-pku.cn/)

on estrogen bioavailability [68]. Thus, both AR agonists and antagonists could be potentially applied as therapeutic agents for breast cancer. A phase II clinical trial has shown that enzalutamide, an AR antagonist approved for treating prostate cancer, improved treatment outcome in AR-positive TNBC patients [69]. MMP9 is a matrix metalloproteinase responsible for breaking down the extracellular matrix (ECM). Its expression is low in normal breast tissues. However, MMP9 is overexpressed in breast cancer cells and promotes breast cancer cell migration and invasion via cleaving E-cadherin and degrading ECM [70, 71]. However, it has been shown that overexpression of MMP9 can trigger the generation and release of endostatin and tumstatin, both of which are capable of suppressing breast cancer progression and metastasis [72, 73]. The expression of MMP9 has been reported to be associated with the presence of AR in epithelial ovarian cancer cells [74]. However, this association is not observed in breast cancer (Fig. 8).

For the two genes that are not differentially expressed in breast cancer patients, gene *AKT1* is a key player of the PI3K-AKT-mTOR signaling pathway in breast cancer although its expression is not statistically significantly increased in breast cancer patients. Activation of the PI3K-AKT-mTOR signaling pathway promotes cell proliferation, growth and survival and induces drug resistance in breast cancer [74, 75]. This pathway has emerged to be one of the major targets in developing therapeutics to treat solid tumors including breast cancer [76]. *SRC* (encoding SRC or c-SRC) is a proto-oncogene that is frequently activated in solid tumors including breast cancer [77]. SRC can activate multiple signaling pathways to promote cancer cell proliferation, growth, survival, migration and invasion [78–80]. Both epidermal growth factor (EGF) and SRC can bind to EGFR and enhance each respective cancer-promoting functions [80–82]. Furthermore, SRC was observed to crosstalk with AR during prostate cancer progression [83].

From the above analyses, it is clear that *S. nigrum* can regulate multiple signaling pathways via the six critical hub proteins and quercetin, cholesterol and 3-epi-beta-sitosterol are likely to be the major active ingredients responsible for those regulatory actions. Molecular docking suggests that the three active ingredients could directly bind to the hub proteins to exert the anticancer activity of *S. nigrum*. Therefore, we may conclude that administration of *S. nigrum* is beneficial for breast cancer patients and the anticancer function of *S. nigrum* is via a network including multiple bioactive compounds and multiple protein targets. Synergistic effects could be achieved from regulating multiple pathways. However, caution should be taken in co-administration of chemotherapy drugs and medicinal

herbs such as *S. nigrum* since the drug-herb interactions are basically unknown. These drug-herb interactions may reduce the therapeutic efficacy of chemotherapy drugs and could even be detrimental to cancer patients. We highly recommend avoiding co-administration of chemotherapy drugs and medicinal herbs unless the co-administration is clinically proved to be safe, or the drug-herb interaction is clearly defined.

5 Conclusion

In this study, we used network pharmacology and molecular docking approaches to illustrate that *S. nigrum* may exert its anticancer function via interactions between the 6 active ingredients and 80 protein targets. Out of these interactions, the interactions between quercetin, cholesterol and 3-epi-beta-sitosterol and 6 hub proteins (AKT1, ESR1, EGFR, SRC1, AR and MMP9) are more important. Synergistic effects are likely to be achieved from the multiple signaling pathways. Briefly, our current research indicates that the anticancer activity of *S. nigrum* is probably due to synergistic effects achieved from multiple bioactive compound-target interactions.

In addition, we would like to point out several limitations of the current study. First, because of the vast amounts of data from the literature, discrepancies may exist in various databases. Secondly, during the decocting process, active compounds with low concentrations may be dismissed and/or unidentified gradients may be generated. Finally, as genes are dynamically regulated by a variety of factors, gene expression information extracted from the databases may not be fully representative for the diseases.Despite several limitations, the current study sets the groundwork for further experimental and clinical confirmation of *S. nigrum*'s molecular mechanism for fighting breast cancer.

Our present approach of determining the MOAs for *S. nigrum* including multi-component, multi-target, and multi-pathway may be also applicable for identifying the MOAs for other medicinal herbs.These medicinal herbs unquestionably merit further pharmacological and clinical research to validate theoretical predictions and standardize their applications in cancer treatment.

Author contributions Conceptualization, YS and JY; methodology, YS; investigation, YS and JY; resources, MKS; data curation, YS; writing—original draft preparation, YS; writing—review and editing, YS and JY; visualization, YS; supervision, JY; funding acquisition, YS. All authors have read and agreed to the published version of the manuscript.

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Data availability The authors do not have permission to share data. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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