

Research article

# Deciphering the Anticancer Mechanism of Naringenin and Berberine in Breast Cancer via Network Pharmacology and Molecular Docking

Shaikh Mo Sarosh, Shaikh Moin, Shaikh Mehmood Dawood\*

Aurangabad Pharmacy College, Mitmita, City- Chhatrapati Sambhajinagar, Maharashtra, Country – India.

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\*Corresponding Author: Shaikh Mehmood Dawood, Aurangabad Pharmacy College, Mitmita, City- Chhatrapati Sambhajinagar, Maharashtra, Country – India.

Email id: mehmoodpharma99@gmail.com

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## Abstract

Breast cancer is one of the most frequent malignancies affecting women globally, and it remains a leading source of cancer-related morbidity and death. Despite major advances in conventional therapy, obstacles such as drug resistance, systemic toxicity, and poor therapeutic selectivity continue to restrict their effectiveness. As a result, the discovery of safer multitarget therapeutic molecules from natural sources has sparked significant scientific interest. The current study used an integrated network pharmacology and molecular docking technique to examine the therapeutic potential of Naringenin and Berberine against breast cancer. SwissTargetPrediction and PubChem were used to identify potential phytochemical targets, while the GeneCards database was searched for breast cancer-associated genes. Protein-protein interaction analysis and pathway enrichment studies revealed critical targets such as SRC, PIK3CA, PIK3CB, PIK3CD, CYP3A4, IGF1R, ESR1, and KDR, which are implicated in oxidative stress control, cell proliferation, endocrine resistance, and cancer progression pathways. Molecular docking research revealed that Naringenin and Berberine had favorable binding interactions with SRC (7A3D) and PIK3CA (2V1Y). Berberine had docking scores of -6.6 kcal/mol against SRC and -6.2 kcal/mol against PIK3CA, whereas Naringenin had scores of -5.8 kcal/mol and -6.9 kcal/mol, respectively, compared to the reference medication Alpelisib. These data point to the multitarget anticancer potential of these phytochemicals against breast cancer.

## 1. Introduction

Breast cancer (BCa) is a major health concern globally, affecting millions of women worldwide. BCa is an important subject for public health interventions. In 2022, about 2.3 million women were diagnosed with BCa, and about 670,000 deaths were also reported from around the world [1]. BCa is the predominant cancer type among women in 157 countries, reflecting its widespread [1]. It is projected that 56,500 cases of ductal carcinoma and 310,720 new cases of invasive BCa will be diagnosed in the US alone in 2024 [2]. The lifetime risk of a woman being diagnosed with BRCA is about 1 in 8, or 13.1%, while the

mortality risk is around 2.3% [2]. Improvements in early detection and treatment have significantly increased survival rates for BCa patients. Currently, localised BCa has a 5-year relative survival rate of 99% [3]. Yet, even as these numbers improve, disparities persist. While Black women's mortality rate is higher than White women's, their incidence rate is lower, which reflects inequities in access to care and treatment outcomes [3]. Natural products have been projected as a possible alternative in the treatment of many diseases due to their myriad bioactive compounds and relatively low toxicity when compared with other pharmaceuticals [4-9]. Natural phytochemicals have received a lot of attention in cancer research because to their

pleiotropic pharmacological properties and generally low toxicity profiles. Among these chemicals, naringenin and berberine have emerged as interesting bioactive molecules with strong anticancer properties. Naringenin, a naturally occurring flavanone found in citrus fruits, has antioxidant, anti-inflammatory, antiproliferative, and pro-apoptotic properties. Naringenin has been shown to decrease breast cancer growth by altering signaling pathways such as PI3K/Akt, NF- $\kappa$ B, MAPK, Wnt/ $\beta$ -catenin, and TGF- $\beta$  [10-12].

Berberine, an isoquinoline alkaloid found in numerous medicinal plants including *Berberis vulgaris* and *Coptis chinensis*, has demonstrated significant therapeutic potential against a variety of malignancies, including breast cancer. Berberine fights cancer by inducing apoptosis, arresting the cell cycle, modulating autophagy, inhibiting epithelial-mesenchymal transition, and suppressing tumor invasion and metastasis. Furthermore, berberine has been shown to affect multiple molecular targets linked to breast cancer growth, including the AKT1, EGFR, MAPK, TP53, VEGFA, and STAT3 signaling pathways [13-15]. Recent breakthroughs in computational biology have made network pharmacology an effective technique for analyzing the complicated relationships between phytochemicals, molecular targets, signaling pathways, and illnesses. Unlike the traditional one drug-one target paradigm, network pharmacology uses a systems-level approach to understand multi-component and multi-target therapeutic processes. This method is especially appropriate for natural chemicals, which frequently have synergistic effects by modulating linked biological networks. Molecular docking enhances network pharmacology by confirming the binding affinities and interaction patterns of bioactive drugs with critical target proteins [16-17].

Several research have individually studied the therapeutic benefits of naringenin and berberine on breast cancer; however, thorough comparative assessments including both drugs within a unified network pharmacology and molecular docking framework are still lacking. The current work intended to extensively analyze the therapeutic potential and molecular mechanisms of naringenin and berberine against breast cancer utilizing integrated *in silico* techniques. This comprehensive computational analysis may give new insights into naringenin and berberine's multi-target therapeutic processes, therefore supporting their future development as possible breast cancer therapies.

## 2. Materials and Methods

### 2.1 Chemical Candidates and Compound Related Targets

The literature was used to aid the chemical selection process. The selected bioactive compounds were then searched in the PubChem database to obtain their chemical structures and SMILES notations. Potential target proteins linked with

these chemicals, with potential scores higher than zero, were then predicted using the Swiss Target Prediction Database.

### 2.2 Identification of Breast Cancer-Related Targets

We searched the GeneCards database for possible targets related with breast cancer. This database combines data from several sources to provide complete information on human genes. The selected targets were collated for additional study and classified as breast cancer-related targets [30].

### 2.3 Venn Diagram

We developed a Venn diagram to assess the overlap between breast cancer-associated targets and possible targets for Naringenin and Berberine.

### 2.4 Protein-Protein Interaction (PPI) Network

The STRING database (<https://string-db.org/>) collects known and projected protein-protein interactions, including functional and physical correlations. For this study, we utilized STRING [30] to look at the connections between the chemicals in our analysis and the targets associated with breast cancer, finding potential therapy targets. To build a reliable protein-protein interaction (PPI) network for these targets, we employed the "Homo sapiens" organism with a confidence level of 0.4. This confidence score is commonly employed because it strikes a good balance between sensitivity and specificity, resulting in a credible criteria for interaction prediction. As a consequence, only proteins with interaction scores of 0.4 or higher were included to the PPI network, increasing the accuracy of our potential target identification.

### 2.5 Functional Enrichment and Pathway Analysis

To identify key pathways and related Gene Ontology (GO) concepts, all potential therapeutic targets were analyzed using pathway and GO enrichment analyses in the String database [30] (<https://string-db.org/>). This investigation shed light on the biological processes (BP), molecular functions (MF), and cellular components (CC) connected with the targets. Pathways and GO keywords having a significance level of  $p < 0.05$  were chosen for further study.

### 2.6 Building a Compound-Target Network

The compound-target interaction network was created by linking the targets associated with each chemical. Cytoscape 3.10.2 (Cytoscape Consortium, San Diego, CA, USA) was used to visualize the network [30]. Nodes in the network represent drugs and their targets, while edges show their interactions.

### 2.7 Molecular Docking

Molecular docking investigations were carried out in accordance with the previously published approach described by Ahmad *et al.* (2024). The three-dimensional structures of target proteins were obtained from the Protein Data Bank, while ligand structures were obtained from

PubChem. AutoDock Vina was used to investigate binding affinity and protein-ligand interactions. The docked complexes with the lowest binding energy were chosen and visualized using PyMOL and Discovery Studio, following the previously defined computational process [22-24].

### 3. Result and Discussion

#### 3.1 Potential Therapeutic Targets of Compounds Used to Treat Breast Cancer

Using breast cancer as a keyword, data were extracted from the GeneCards database, providing 20521 target genes. Among these, 171 targets were identified as shared by the chemicals and breast cancer-related genes, indicating prospective therapeutic targets for compounds used to treat breast cancer. A Venn diagram (Figure 1) depicts these overlapping targets.

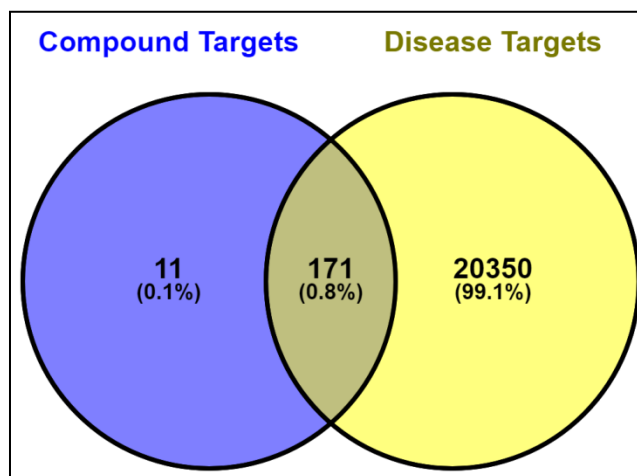


Figure 1. Venn Diagram.

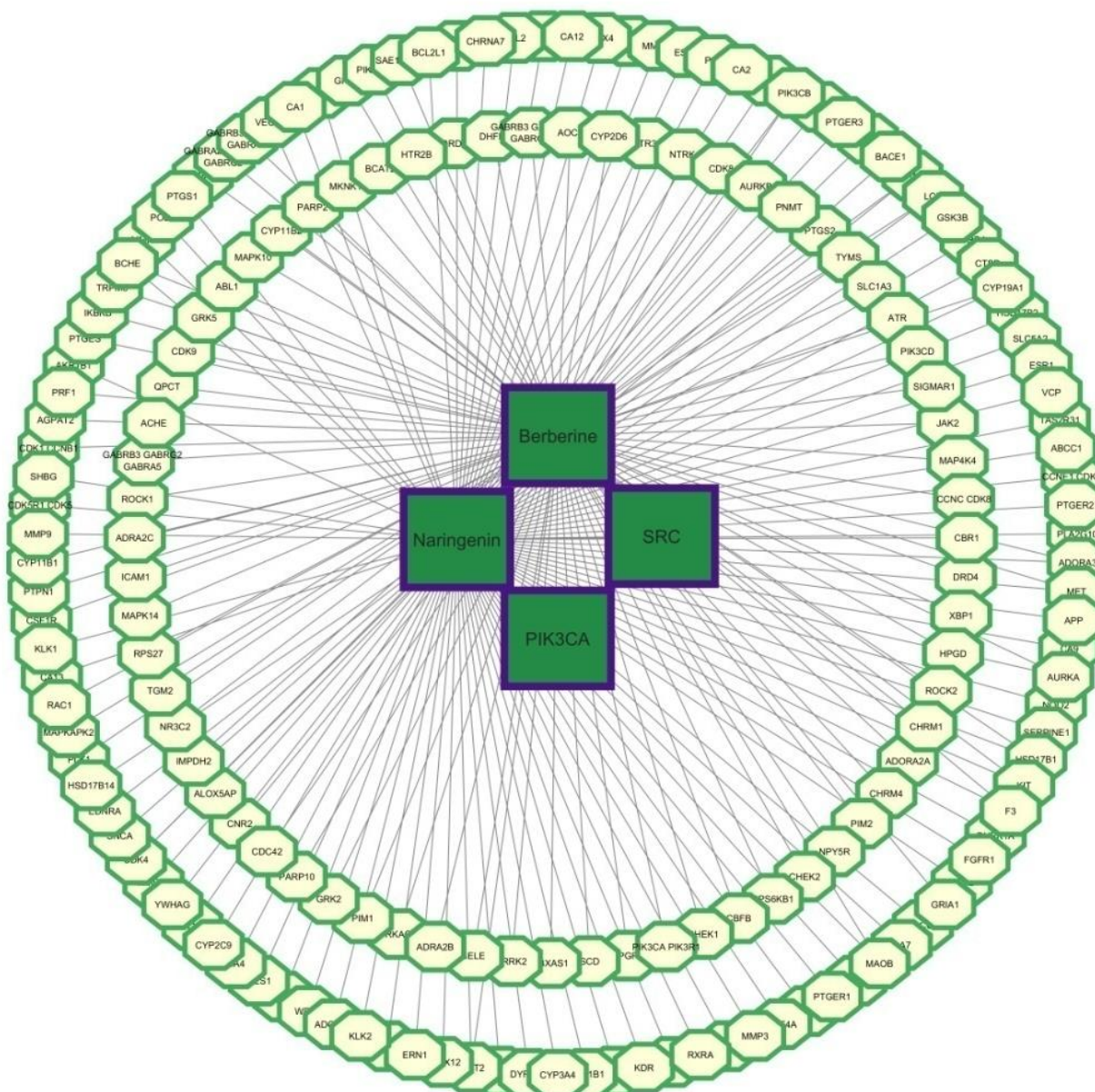


Figure 2. Compound Target Network.

### 3.2 Construction of the Compound-Target Network

To investigate the signaling pathways and functional functions of the identified target genes, we used Cytoscape data analysis. This strategy made it easier to create a sophisticated compound-target network, as shown in Figure 2. The network highlights the pharmacological pathways by which the chemicals may impact breast cancer therapy, with a focus on interactions with 197 target proteins. Network analysis shows that different components are converging across numerous targets, implying that these bioactive chemicals may have synergistic effects. These interactions may improve the therapeutic efficacy of these drugs not just in the treatment of breast cancer, but also in other disorders.

### 3.3 PPI Network Visualization and Analysis

The STRING database was used to investigate interactions between 171 putative medicinal targets. The protein-protein interaction (PPI) network included 171 nodes and 184 edges, an average node degree of 2.15, and a local clustering coefficient of 0.427 (Figure 3). In this network, nodes represent individual proteins, while edges represent the relationships between them. A higher degree value indicates a more important involvement for the protein in the network. Several hub genes were discovered after filtering based on recognized criteria, including SRC, PIK3CA, PIK3CB, PIK3CD, CYP3A4, IGF1R, AKR1C3, ESR1, KDR, and CYP19A1. These proteins, which include biological

enzymes and cytokines, play critical roles in a variety of regulatory processes such as signal transduction and protein phosphorylation. Among these genes, the top five nodal targets were SRC, PIK3CA, PIK3CB, PIK3CD, and CYP3A4. The substantial connections between these genes and other possible therapeutic targets highlight their relevance in breast cancer therapy.

### 3.4 GO Enrichment Analyses

To further understand the molecular processes by which drugs affect breast cancer, we performed Gene Ontology (GO) enrichment analysis on 171 possible therapeutic targets linked with these compounds using the string database. This study divided into three categories: biological process (BP), molecular function (MF), and cellular component. The top ten GO keywords found in each category are displayed in a bar plot, with data described in Tables 1 (BP), Table 2 (MF), and Table 3 (CC), as well as Figure 4A, 4B, and 4C. In the visualizations, the size of each bar correlates to the number of enriched genes linked with that GO word, while the color gradient indicates the importance of the p value, with deeper colors representing lower p values. A wider bar indicates a greater number of enriched therapeutic genes inside the associated GO word, implying a stronger link between that phrase and breast cancer therapy than the other terms.

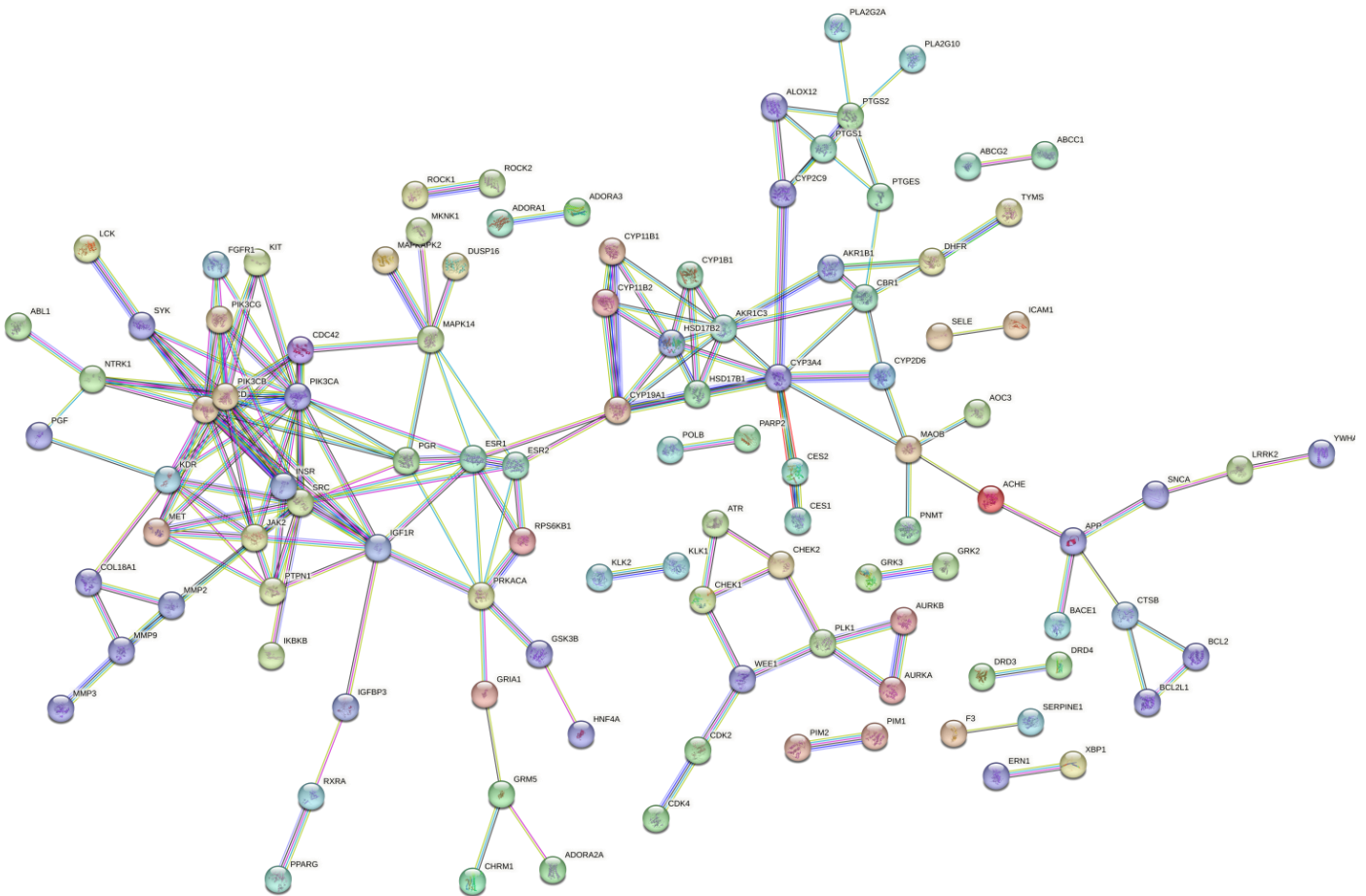


Figure 3. PPI Network.

### 3.5 KEGG Pathway Enrichment Analyses

The pathways associated with potential therapeutic targets for breast cancer treatment were identified through KEGG pathway enrichment analysis. Signaling pathways were obtained via the string database. The top 10 signaling

pathways were then visualized in a bar graph (Table 4 and Figure 5), organized by their P values in ascending order. The analysis indicated that the key targets were notably enriched in the Endocrine Resistance pathway (Figure 5A) and the RAS Signaling pathway (Figure 5B).

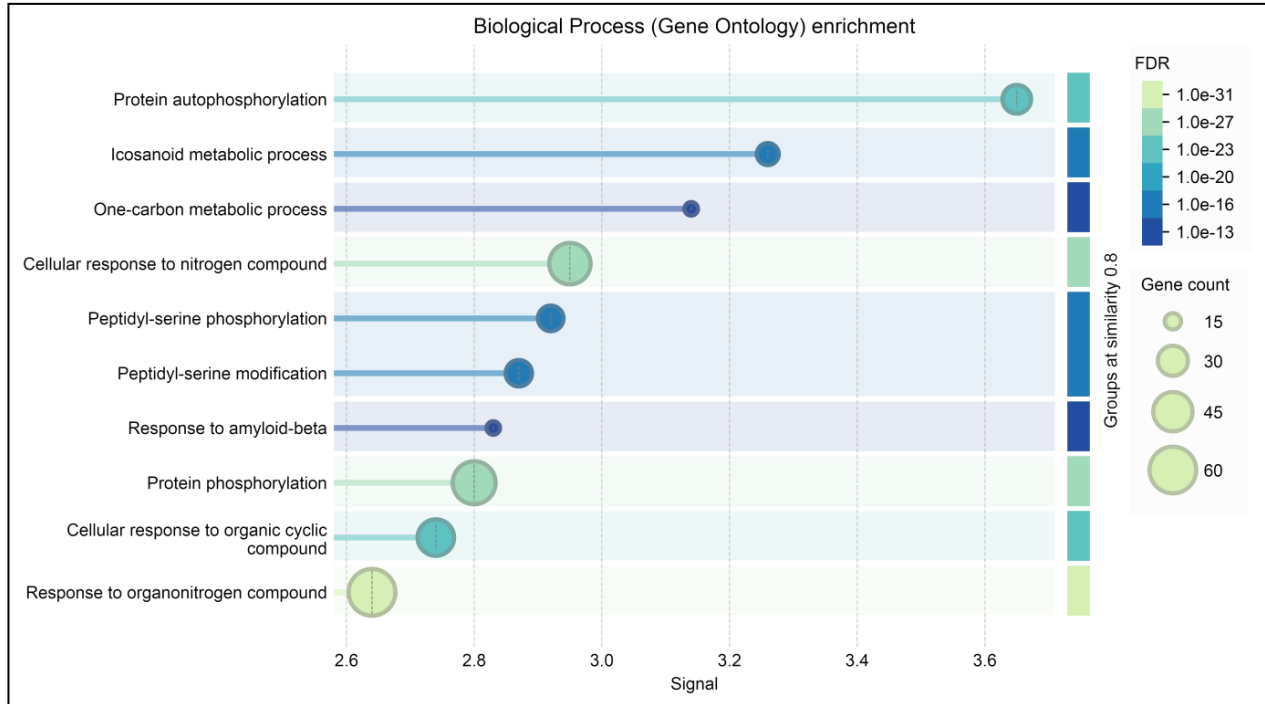


Figure 4A. Biological Process.

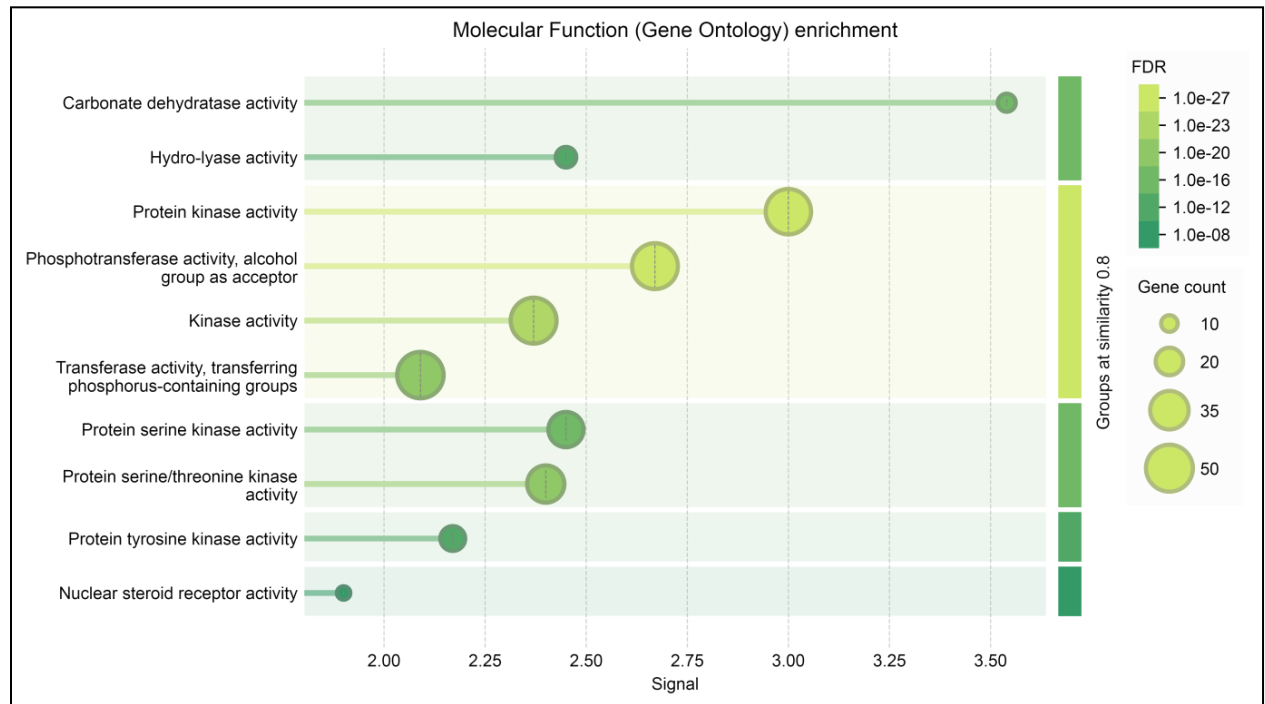


Figure 4B. Molecular Function.

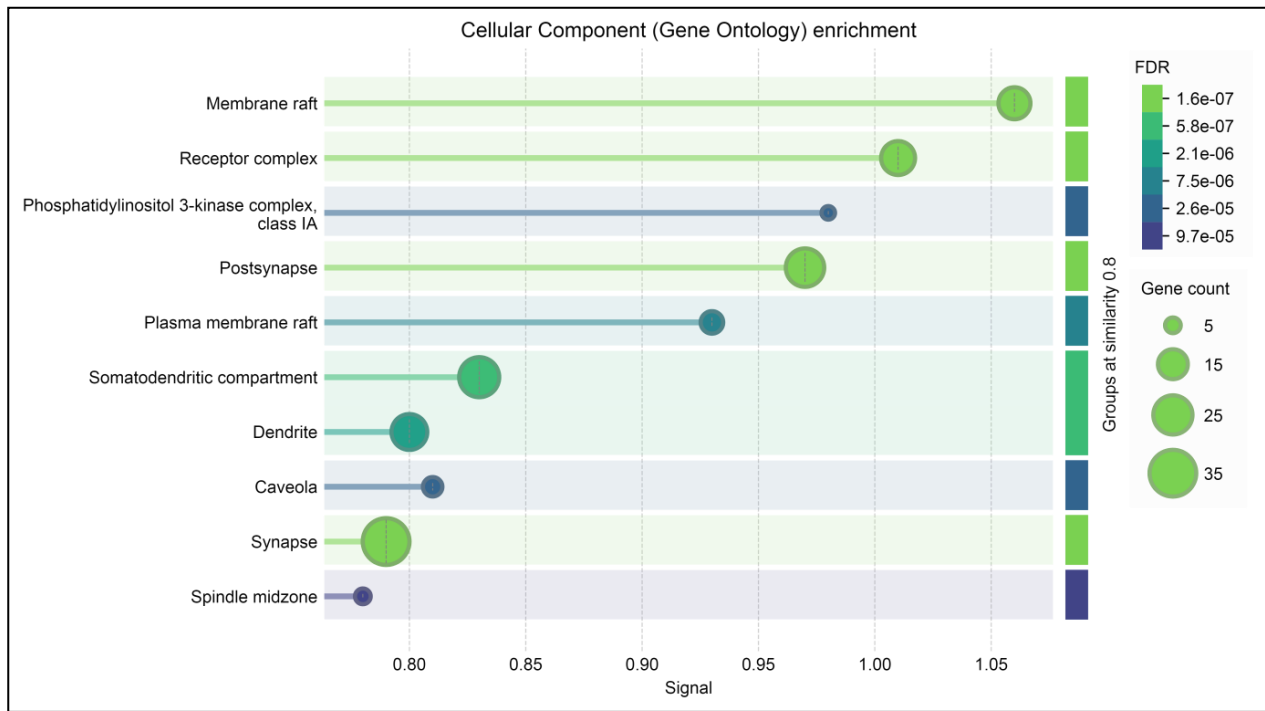


Figure 4C. Cellular Components.

Table 1. Biological Process.

Description	Observed gene count	False discovery rate
Response to chemical	126	7.73E-46
Response to oxygen-containing compound	84	1.07E-41
Response to organic substance	103	4.89E-41
Cellular response to chemical stimulus	101	1.88E-40
Response to nitrogen compound	64	1.76E-32
Response to stimulus	147	4.70E-32

Table 2. Molecular Function.

Description	Observed gene count	False discovery rate
Catalytic activity	122	9.87E-28
Protein kinase activity	47	1.35E-27
Ion binding	125	1.26E-26
Phosphotransferase activity, alcohol group as acceptor	48	7.97E-26
Kinase activity	48	1.46E-23
Transferase activity, transferring phosphorus-containing groups	49	1.46E-21

Table 3. Cellular Components.

Description	Observed gene count	False discovery rate
Cytoplasm	143	3.57E-07
Postsynapse	24	1.65E-06
Intrinsic component of plasma membrane	42	1.72E-06
Plasma membrane	83	2.89E-06
Integral component of plasma membrane	40	2.89E-06
Endomembrane system	75	2.89E-06

Table 4. KEGG Pathway.

Description	Observed gene count	False discovery rate
Endocrine resistance	15	1.91E-12
Ras signaling pathway	21	2.59E-13
Pathways in cancer	36	3.71E-19
PI3K-Akt signaling pathway	25	2.07E-13
Proteoglycans in cancer	17	1.64E-10
EGFR tyrosine kinase inhibitor resistance	12	5.58E-10

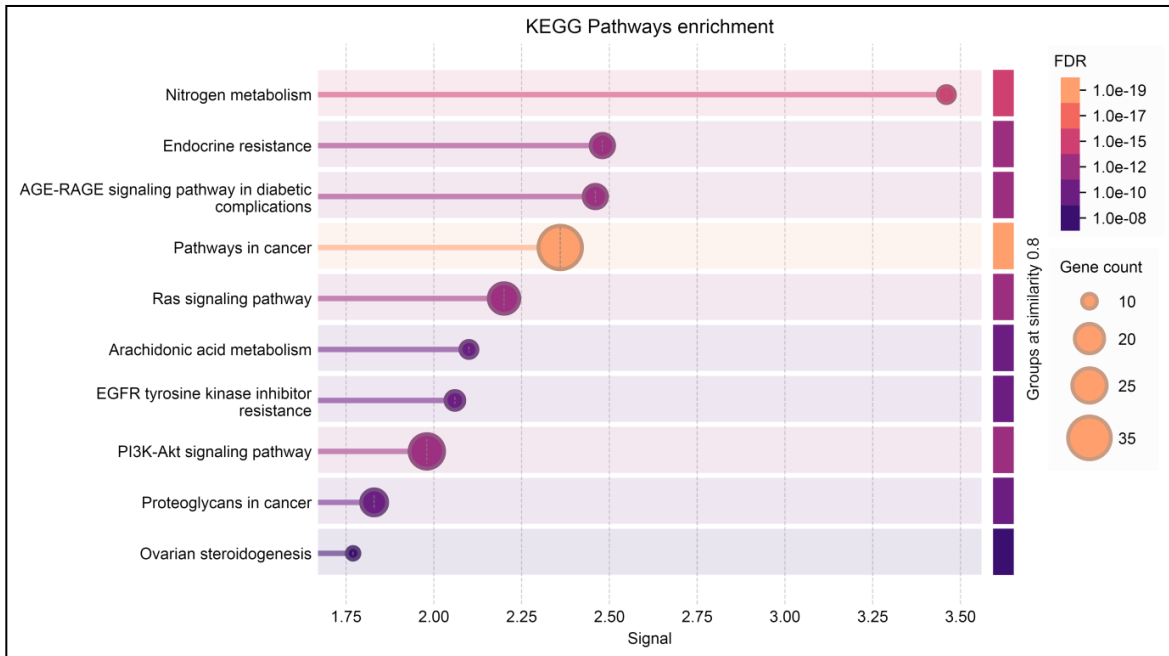


Figure 5. KEGG Pathway.

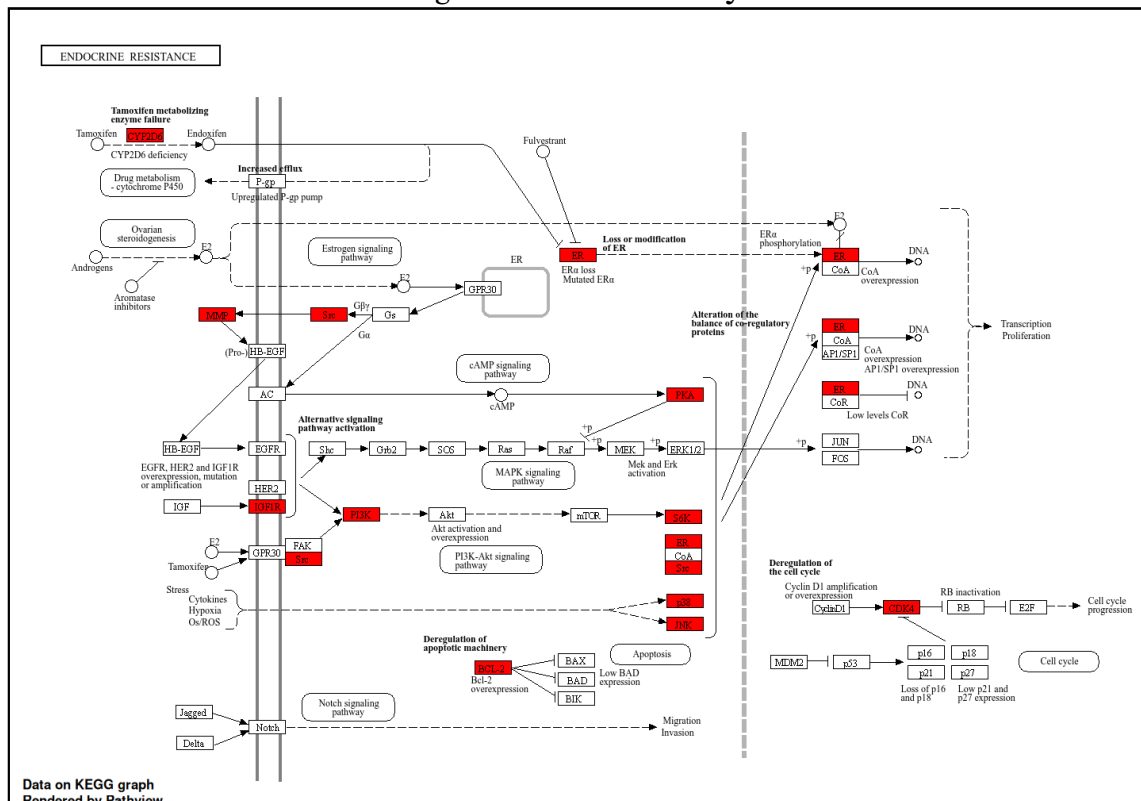


Figure 5A. Endocrine Resistance Pathway.

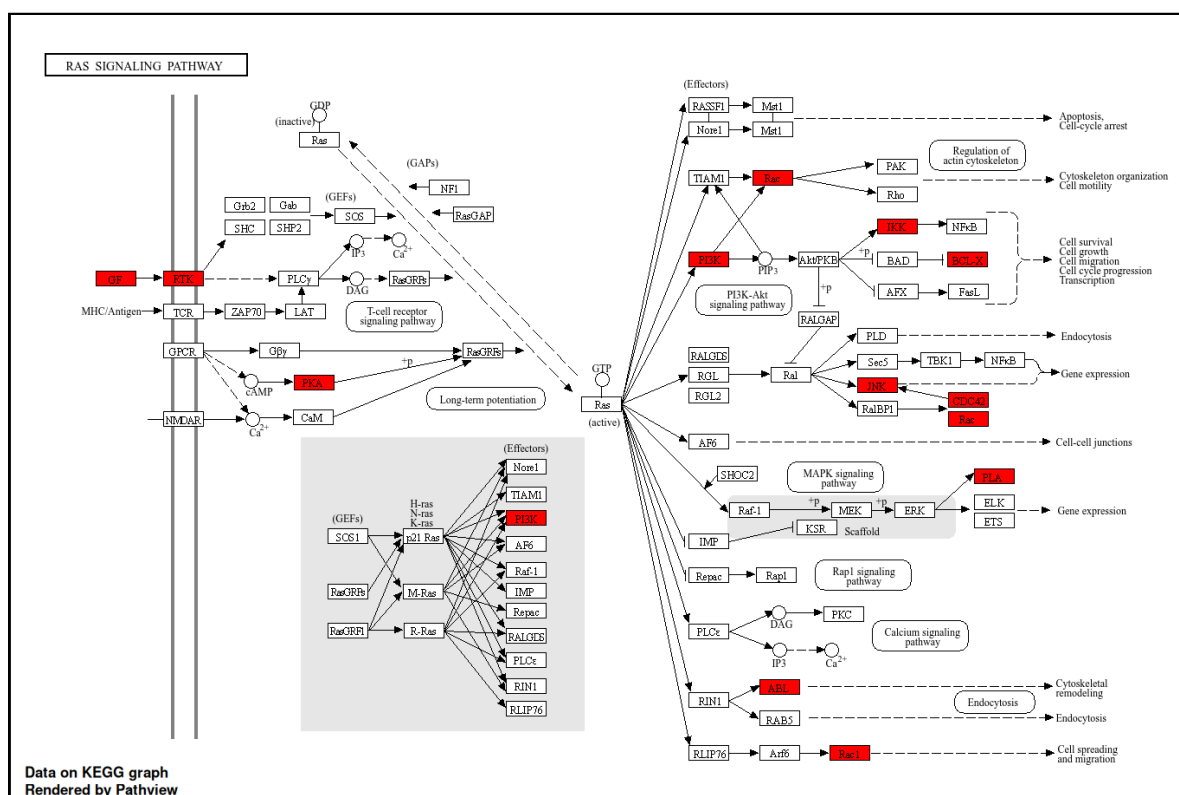


Figure 5B. RAS Signaling Pathway.

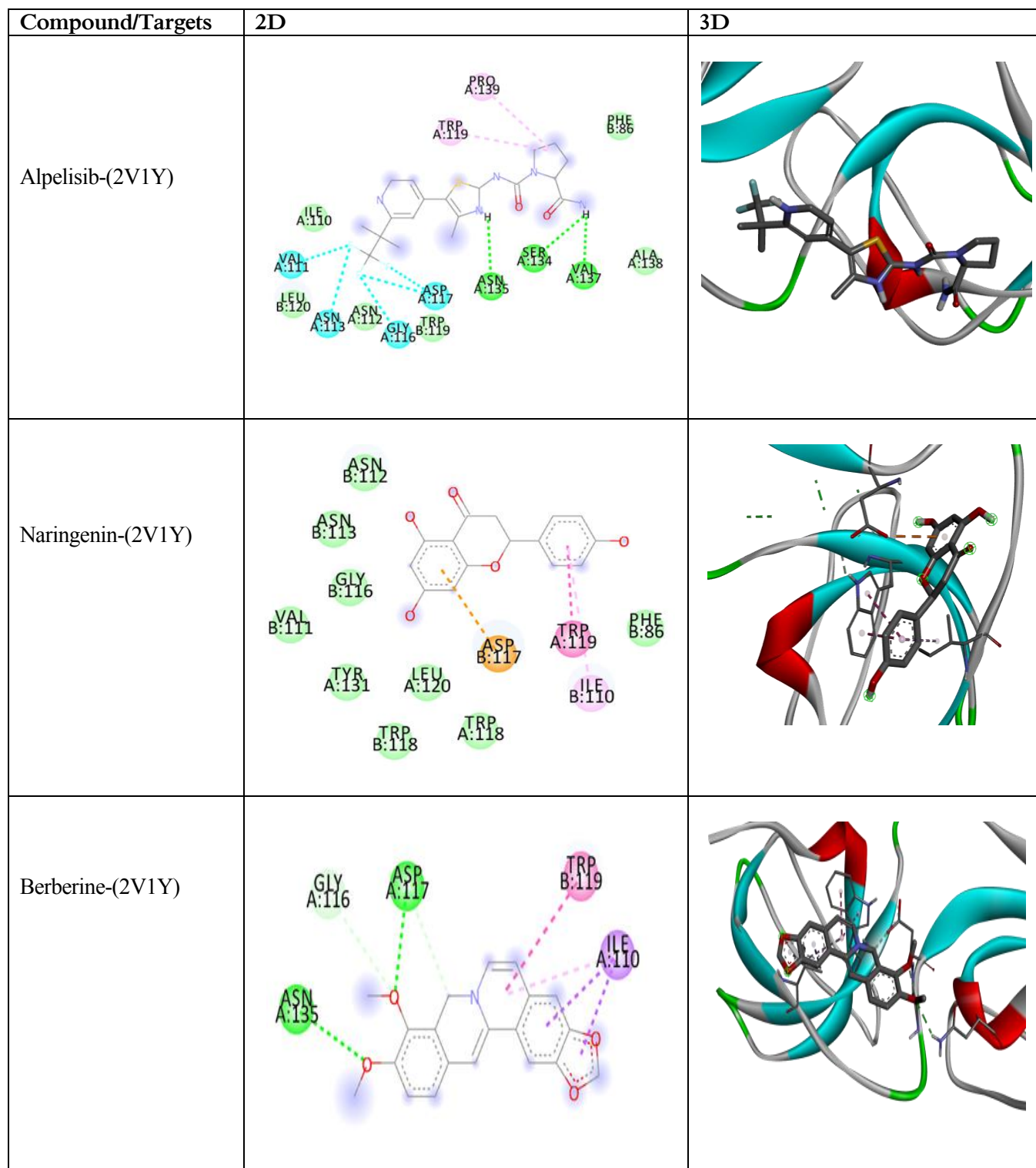
### 3.6 Molecular docking

Molecular docking (MD) studies of Naringenin and Berberine with key breast cancer-associated targets, including SRC (node degree = 17) and PIK3CA (node degree = 16), were performed using AutoDock Vina to evaluate binding affinities and interaction profiles. The

docking analysis assessed parameters include binding affinity (kcal/mol), interacting amino acid residues. The obtained docking results demonstrated favorable ligand-protein interactions and are summarized in Table 5 and illustrated in Figure 6.

Table 5. Molecular docking analysis.

Sr. No.	Compound	Target Protein	PDB ID	Binding Energy (kcal/mol)	Interpretation
1	Alpelisib	SRC Proto-oncogene Kinase	7A3D	-7.0	Demonstrated strong binding affinity toward SRC target protein.
2	Naringenin	SRC Proto-oncogene Kinase	7A3D	-5.8	Exhibited moderate binding interaction with SRC protein.
3	Berberine	SRC Proto-oncogene Kinase	7A3D	-6.6	Showed considerable binding affinity against SRC protein.
4	Alpelisib	Phosphatidylinositol-4,5-bisphosphate 3-kinase subunit alpha (PIK3CA)	2V1Y	-7.1	Exhibited the highest binding affinity among tested compounds against PIK3CA protein.
5	Naringenin	Phosphatidylinositol-4,5-bisphosphate 3-kinase subunit alpha (PIK3CA)	2V1Y	-6.9	Demonstrated strong interaction and stable binding with PIK3CA target protein.
6	Berberine	Phosphatidylinositol-4,5-bisphosphate 3-kinase subunit alpha (PIK3CA)	2V1Y	-6.2	Showed moderate binding affinity toward PIK3CA protein.



**Figure 6. Illustrates 2D and 3D docking poses of Alpelisib, Naringenin and Berberine with protein PIK3CA (2V1Y).**

Molecular docking analysis was performed to evaluate the binding affinity of the selected compounds against important breast cancer-associated target proteins including SRC proto-oncogene tyrosine kinase (PDB ID: 7A3D) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) (PDB ID: 2V1Y). The docking results demonstrated favorable binding interactions of all

tested compounds with the selected target proteins, indicating their potential therapeutic relevance in breast cancer management. Among the tested compounds, Alpelisib exhibited the strongest binding affinity against both target proteins with binding energies of  $-7.0$  kcal/mol toward SRC (7A3D) and  $-7.1$  kcal/mol toward PIK3CA (2V1Y). The strong binding affinity observed for Alpelisib

may be attributed to its established inhibitory activity against PI3K-associated signaling pathways involved in tumor growth, proliferation, survival, and metastasis in breast cancer. The docking findings further validate the suitability of the selected targets for computational screening studies. Naringenin demonstrated considerable binding affinity against PIK3CA with a docking score of -6.9 kcal/mol, while showing moderate interaction with SRC protein (-5.8 kcal/mol). The observed interactions suggest that Naringenin may interfere with PI3K/Akt-mediated oncogenic signaling pathways associated with breast cancer progression. Previous studies have also reported antioxidant, antiproliferative, and apoptosis-inducing activities of Naringenin against various cancer cell lines, supporting the present docking observations.

Similarly, Berberine showed notable binding affinity toward SRC (-6.6 kcal/mol) and moderate interaction with PIK3CA (-6.2 kcal/mol). The interaction profile indicates the potential of Berberine to modulate tyrosine kinase-mediated signaling pathways involved in cancer cell proliferation and survival. The binding energies obtained in the present study suggest stable ligand-protein complex formation and possible inhibitory activity against breast cancer-associated molecular targets. The molecular docking results indicate that the selected phytochemicals possess promising interaction potential against important proteins implicated in breast cancer pathogenesis. The strong binding affinity observed particularly against PIK3CA and SRC proteins suggests that these compounds may contribute to modulation of oncogenic signaling pathways including PI3K/Akt and SRC-associated pathways involved in tumor progression, angiogenesis, and metastasis. These findings provide a computational basis for further experimental validation and development of phytochemical-based therapeutic strategies against breast cancer.

#### 4. Conclusion

The present research used integrated network pharmacology and molecular docking studies to demonstrate whether Naringenin and Berberine had multitarget therapeutic potential against breast cancer. Key targets and signaling pathways linked to oxidative stress, cell proliferation, endocrine resistance, and cancer development were discovered. Molecular docking experiments demonstrated that both phytochemicals had favorable binding interactions with SRC kinase and PIK3CA proteins, suggesting their potential anticancer effect. Berberine had a high binding affinity for SRC, whereas Naringenin interacted significantly with PIK3CA. These findings indicate that Naringenin and Berberine may be attractive natural alternatives for breast cancer treatment and provide a scientific foundation for future experimental and clinical studies.

#### Author Contribution

Shaikh Mo Sarosh carried out the literature survey, network pharmacology analysis, and manuscript preparation. Shaikh Moin performed molecular docking analysis and data interpretation. Dr. Shaikh Mehmood Dawood designed and supervised the study, reviewed the manuscript, and approved the final version for publication.

#### Funding Statement

The authors declare that no financial support or funding was received for this study.

#### Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

#### Ethics Approval and Consent to Participate

This study was entirely based on computational and *in silico* methodologies including network pharmacology and molecular docking approaches. Therefore, ethical approval and informed consent were not required as no human participants or experimental animals were involved in the study.

#### Consent for Publication

All authors have read and approved the final version of the manuscript for publication.

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#### Declaration of Generative AI

During the preparation of this manuscript, generative artificial intelligence (AI)-assisted tools were utilized only for language refinement, grammatical correction, and improvement of scientific readability.

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