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Article

Integrative Network Pharmacology and Molecular Docking Analysis of *Tinospora crispa* L. for Breast Cancer Therapy

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Abstract

Breast cancer remains one of the leading causes of mortality related to cancer. Therefore, it is necessary to explore novel therapeutic approaches, particularly those using plant-derived natural products. *Tinospora crispa* L. contains diverse bioactive compounds with reported antioxidant and anticancer activities. This study integrates network pharmacology and molecular docking approaches to elucidate the molecular mechanisms of *T. crispa* against breast cancer. A total of 72 overlapping targets were identified between *T. crispa* compounds and breast cancer-related proteins, forming a highly interconnected PPI network consisting of 72 nodes and 340 edges. Clustering and enrichment analyses revealed that the second cluster was significantly associated with cancer-related pathways, including AKT1, MAPK, and EGFR. The compound–protein–pathway network showed that two compounds from *T. crispa,* apigenin and cycloeucalenone, had the highest degrees, suggesting central regulatory roles. Molecular docking analysis indicated that cycloecalenone exhibited the strongest predicted binding affinity toward EGFR (-9.6 kcal/mol) and AKT1 (9.8 kcal/mol), predominantly mediated by hydrophobic interaction. In contrast, apigenin demonstrated a more favorable interaction with MAPK3 (-9.1 kcal/mol), stabilized by multiple hydrogen bonds and hydrophobic interactions. These findings indicate that *T. crispa* compounds may exert anticancer effects by modulating multiple oncogenic pathways, supporting their potential development as multi-target agents in breast cancer therapy

Keywords: *Breast cancer, molecular docking, network pharmacology, Tinospora crispa.*

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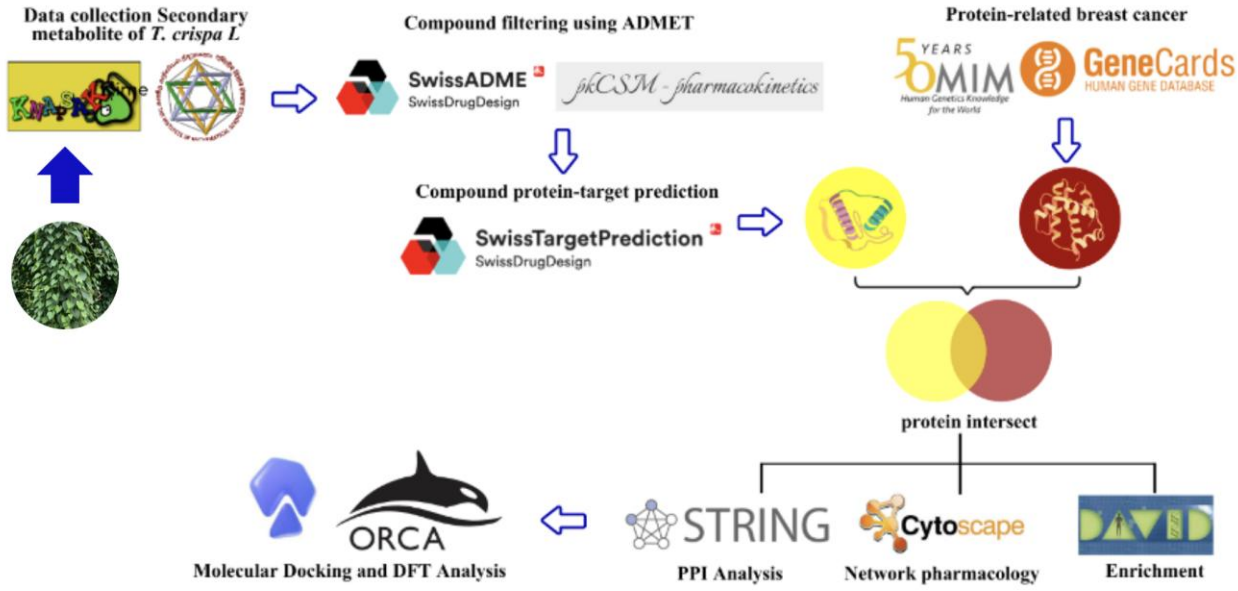
Graphical Abstract



Introduction

Wounds Cancer is one of the deadliest diseases in the world and remains a challenge to the health system. Global Cancer Observatory (GLOBOCAN) reported that more than 19 million new cancer cases and nearly 10 million cancer-related deaths were reported in 2019 [1]. Radiotherapy and chemotherapy have been used as primary treatment, but they are still costly and accompanied by negative side effects to the patient's body [2]. Therefore, finding an alternative treatment other than these two remains challenging.

Natural products are an attractive alternative for developing cancer drugs based on secondary metabolites. This is due to their favorable bioavailability, lower toxicity, and broad availability, including commonly consumed foods. Several classes of secondary metabolites have been reported to have anticancer potential, namely alkaloids and flavonoids [3], [4]. *Tinospora crispa*, a local herbal plant, is among the herbs with potential anticancer activity. Several scientists have reported that *T. crispa* extracts exhibit antidiabetic, antihypertensive, wound-healing, and antimalarial properties. Furthermore, phytochemical analysis revealed the presence of several secondary metabolites, including phenolics, flavonoids, alkaloids, and saponins, which exhibit antioxidant, anti-inflammatory, and anticancer activities [5-8]. However, the complexity of the extract contained in *T. crispa* still presents a challenge in analyzing which compounds contribute as active compounds and the mechanism of interaction between active compounds and related proteins. Therefore, A network pharmacology approach can overcome this problem.

Network pharmacology is an analysis used to explore the mechanism of interaction between drug candidates or secondary metabolites, in the context of biological networks, such as protein-protein interaction (PPI), compound-target network, and metabolic-disease pathways, and to predict new potential targets or protein targets toward specific diseases [9-11]. The results of network pharmacology analysis can be used to accept, reject, or determine

whether to proceed to the preclinical stage. The advantage of this method is that it accelerates research, cuts costs, and reduces risk in drug discovery.

Not only network pharmacology, but also molecular docking is a powerful computational method that helps determine the interaction between a compound and a specific protein. Furthermore, this method identifies the positions of ligands within the active site and characterizes non-covalent interactions, including hydrogen bonds, van der Waals forces, and electrostatic interactions. The resulting binding affinity provides an initial estimate of the strength and stability of these interactions [12-14].

In this study, network pharmacology was used to evaluate the secondary metabolites of *T. crispa* and their mechanisms of action against breast cancer. Following that, overlapping genes related to breast cancer and protein targets of the selected compound were identified and analysed using network pharmacology and enrichment analysis. Finally, the most potent compounds are identified using molecular docking for selected proteins or genes. The full workflow is depicted in Figure 1.

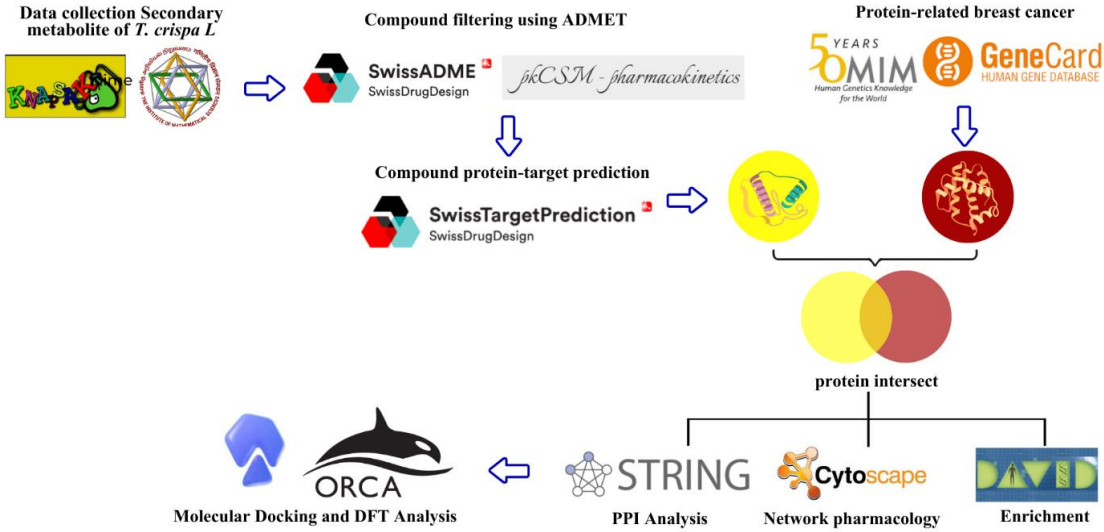


Figure 1. Workflow study of *T. crispa L.* as Breast Cancer Therapy

Materials and Methods

Hardwares and Softwares

This study was conducted using *in silico* approaches, including network pharmacology, ADMET prediction, and molecular docking analyses. The computational analyses were performed on a laptop installed with an Intel® Core i5-7200U CPU, 8GB RAM, and 2 GB VRAM. The software utilized in this study included MarvinSketch for structure drawing and harvesting SMILES. Cytoscape for network visualization and analyses, Microsoft Excel 365 for data processing, Autodock Tool 1.5.6 and AutoDock Vina for molecular docking, and BIOVIA Discovery Studio Visualizer for visualizing docking results.

Preparation of Tinospora crispa L. active compound, ADMET, and Druglikeness

The active compound of *Tinospora crispa* was retrieved from the Knapsack (https://www.knapsackfamily.com/) and the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT)

(https://cb.imsc.res.in/imppat/) database. The chemical structure and SMILES representations of the selected compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and MarvinSketch (academic license). ADMET Analysis was conducted through SwissADME (http://www.swissadme.ch/index.php) and the toxicity analysis web (https://biosig.lab.uq.edu.au/pkcsm/prediction) and (https://lmmd.ecust.edu.cn/admetsar1/predict/). The identified compounds were screened based on pharmacokinetic and toxicity parameters prior to docking analysis. The bioavailability threshold was set at > 0.55. Drug-likeness evaluation followed Lipinski's rule of five, and compounds with more than one violation were excluded. Toxicity profiling included carcinogenicity, AMES mutagenicity, and hepatotoxicity prediction. Compounds predicted to exhibit any of these toxicological risks were removed. The remaining compounds were considered eligible for molecular docking studies.

Protein Target Prediction and Breast Cancer-related Protein Identification

The potential protein targets of each compound were predicted using SwissTargetPrediction (http://www.swisstargetprediction.ch/) and the SEA Search Server (http://sea.bkslab.org/). *Homo sapiens* was selected as the reference species. Proteins associated with breast cancer were retrieved from the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/) and Gene Cards (https://www.genecards.org/). Subsequently, the consensus targets between compound-derived targets and Breast Cancer-associated proteins were identified and subjected to further network analysis. This intersecting protein was considered a potential therapeutic target of the selected compound. These proteins were then prepared for downstream analysis, including protein-protein interaction (PPI) network, pathway enrichment, and KEGG analysis.

Protein-protein Interaction (PPI) Analysis

The Searching Tools STRING (https://string-db.org/) was used to construct the PPI network. Intersection proteins from the previous step were used as input data to form the PPI network. *Homo sapiens* was used as a reference organism. The PPI network was constructed with the highest confidence level (0.9) as the confidence threshold. The results were downloaded and visualized using Cytoscape 3.10.4. In this network, proteins are presented as a node while the interaction between proteins is denoted as an edge.

Gene Ontology and KEGG Enrichment Analysis

Gene Ontology (GO) enrichment analysis was performed using the DAVID Bioinformatics Resource server (https://davidbioinformatics.nih.gov). GO enrichment analysis included biological process (BP), molecular functions (MF), cellular component (CC), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Molecular Docking of Compound-Protein Target

Molecular docking was conducted using specific docking by targeting specific binding sites of the selected protein. Compounds and proteins were selected by constructing a compound-protein pathway and choosing those with the highest degree. Autodock Tool 1.5.6 and AutoDock Vinawas used to conduct molecular docking

[15-16]. Target proteins for molecular docking were prioritized based on topological analysis of the PPI network in Cytoscape. Proteins with the highest centrality were selected as core targets: MAPK3, AKT1, and EGFR. The three-dimensional crystal structures were obtained from the Protein Data Bank: AKT1 (PDB ID: 4GV1), MAPK3 (PDB ID: 4QTB), and EGFR (PDB ID: 1M17). Molecular docking was performed using AutoDock Vina. The grid box parameters were defined to encompass the active binding pocket of each protein. For AKT1, the grid center was set at −20.286, 3.748, 11.736 with dimensions of 40 × 40 × 40 Å For MAPK3, the grid center was positioned at 34.977, 56.722, 50.362 with dimensions of 46 × 40 × 40. For EGFR, the grid center was defined at 21.697, 0.303, 52.093, with dimensions of 42 × 40 × 40. The exhaustiveness is set at 64 with 10 num mode to increase the molecular bonding accuracy. The 2D-interaction was identified using Biovia Discovery Studio.

Result and Discussion

Screening Active Compound through ADMET analysis.

A total of 45 compounds were found from the Knapsack and IMPPAT databases related to T. crispa secondary metabolites. All compound data is available in Table 1. The compounds found are known to belong to several groups, namely alkaloids, terpenoids, terpenoid-glucoside, phenolic, flavonoid, and glycoside. In the next step, the compounds are filtered to obtain selected compounds using bioavailability score (more than 0.55), drug likeness score (more than 0.21) and Lipinski rule of five which include molecular weight is less than 500 Daltons, hydrogen bond donor fewer or equal to 5, hydrogen bond acceptor is fewer 10 or fewer, and the partition coefficient are 5 or less [17]. These fifteen compounds were then screened based on toxicity, including carcinogenicity, AMES (mutagenic potential), and hepatotoxicity. Based on that screening, 10 compounds, 2 of which is enantiomer, were selected for target protein analysis. The details of screening results and the ten selected compounds were available in Table 2.

Table 1. Secondary metabolites from *T. crispa* and screening results-based bioavailability, drug likeness, and Lipinski violation

Compound	Chemical Formula	Molecular weight	Bioavailability scores	Drug-likeness	Lipinski violation
Lemmasterone	$C_{29}H_{48}O_7$	508.69	0.17	1,33	2
Tembetarine	$C_{20}H_{26}NO_4^+$	344.42	0.55	1,21	0
Higenamine	$C_{16}H_{17}NO_3$	271.31	0.55	0,78	0
(-)-beta-Sitosterol	$C_{29}H_{50}O$	414.71	0.55	0,78	1
Magnoflorine	$C_{20}H_{24}NO_4^+$	342.41	0.55	0,78	0
Clionasterol	$C_{29}H_{50}O$	414.71	0.55	0,78	1
Rumphioside F	$C_{28}H_{36}O_{13}$	580.58	0.17	0,76	2
Borapetoside E	$C_{27}H_{36}O_{11}$	536.57	0.17	0,67	2
beta-Stigmasterol	$C_{29}H_{48}O$	412.69	0.55	0,62	1
Litcubinine	$C_{18}H_{20}NO_4^+$	314.36	0.55	0,57	0
borapetoside D	$C_{33}H_{46}O_{16}$	698.71	0.17	0,52	3

Compound	Chemical Formula	Molecular weight	Bioavailability scores	Drug-likeness	Lipinski violation
Rumphioside I	$C_{28}H_{36}O_{12}$	564.58	0.17	0,50	2
Rumphioside C	$C_{29}H_{44}O_{15}$	632.65	0.17	0,47	3
Caffeoyl tyramine	$C_{17}H_{17}NO_4$	299.32	0.55	0.44	0
Cycloeucalenone	$C_{30}H_{48}O$	424.7	0.55	0.42	1
Apigenin	$C_{15}H_{10}O_5$	270.24	0.55	0.39	0
5,4'-Dihydroxy-7-methoxyflavone	$C_{16}H_{12}O_5$	284.26	0.55	0.37	0
Rumphioside B	$C_{28}H_{38}O_{13}$	582.59	0.17	0.36	2
Rumphioside A	$C_{27}H_{36}O_{13}$	568.57	0.17	0.30	2
Cycloeucalenol	$C_{30}H_{50}O$	426.72	0.55	0.25	1
N-Acetylnornuciferine	$C_{20}H_{21}NO_3$	323.39	0.55	0.22	0
N-trans-Feruloyltyramine	$C_{18}H_{19}NO_4$	313.35	0.55	0.21	0
N-cis-Feruloyltyramine	$C_{18}H_{19}NO_4$	313.35	0.55	0.21	0
Moupinamide	$C_{18}H_{19}NO_4$	313.35	0.55	0.21	0
Borapetoside B	$C_{27}H_{36}O_{12}$	552.57	0.17	0.17	2
Cordifolide A	$C_{28}H_{38}O_{12}S$	598.66	0.17	0.16	2
Borapetoside A	$C_{26}H_{34}O_{12}$	538.54	0.17	0.15	2
Borapetoside	$C_{26}H_{34}O_{12}$	538.54	0.17	0.15	2
Borapetoside C	$C_{27}H_{36}O_{11}$	536.57	0.17	0.10	2
Tinocrisposide	$C_{27}H_{36}O_{11}$	536.57	0.17	0.10	2
Diosmetin	$C_{16}H_{12}O_6$	300.26	0.55	0.06	0
Syringin	$C_{17}H_{24}O_9$	372.37	0.55	0.05	0
Borapetoside F	$C_{27}H_{34}O_{11}$	534.55	0.17	-0.12	2
methyl (6S,6aR,10aS,10bS)-2-(furan-3-yl)-6a,10b-dimethyl-4-oxo-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,2,6,9,10,10a-hexahydrobenzo[f]isochromene-7-carboxylate	$C_{27}H_{34}O_{11}$	534.55	0.17	-0.12	2
N-trans-Coumaroyltyramine	$C_{17}H_{17}NO_3$	283.32	0.55	-0.15	0
Tinocrispol A	$C_{21}H_{24}O_7$	388.41	0.55	-0.35	0
Borapetol A	$C_{20}H_{24}O_7$	376.4	0.55	-0.38	0
(-)-Secoisolariciresinol	$C_{20}H_{26}O_6$	362.42	0.55	-0.40	0
N-Formylanonaine	$C_{18}H_{15}NO_3$	293.32	0.55	-0.45	0
Columbin	$C_{20}H_{22}O_6$	358.39	0.55	-0.52	0
Borapetol B	$C_{21}H_{26}O_7$	390.43	0.55	-0.55	0
(-)-Salsolinol	$C_{10}H_{13}NO_2$	179.22	0.55	-0.55	0
Lysicamine	$C_{18}H_{13}NO_3$	291.3	0.55	-0.56	0

Compound	Chemical Formula	Molecular weight	Bioavailability scores	Drug-likeness	Lipinski violation
Tyramine	$C_8H_{11}NO$	137.18	0.55	-0.91	0
Sterol	$C_{17}H_{28}O$	248.4	0.55	-1.07	1

Table 2. Toxicity analysis of selected compounds

Compound	Main Class	Carcinogenicity	AMES	Hepatoxicity	Results
Tembetarine	Alkaloid	No	No	Yes	-
Higenamine	Alkaloid	No	No	No	Ö
(-)-beta-Sitosterol	Steroid	No	No	No	Ö
Magnoflorine	Alkaloid	No	No	Yes	-
Clionasterol	Terpenoid	No	No	No	Ö
beta-Stigmasterol	Steroid	No	No	No	Ö
Litcubinine	Alkaloid	No	No	No	Ö
Caffeoyl tyramine	Phenolic	No	No	Yes	
Cycloeucalenone	Terpenoid	No	No	No	Ö
Apigenin	Flavonoid	No	No	No	Ö
5,4'-Dihydroxy-7-methoxyflavone	Flavonoid	Yes	No	No	-
Cycloeucalenol	Terpenoid	No	No	No	Ö
N-Acetylnornuciferine	Alkaloid	Yes	No	Yes	-
N-trans-Feruloyltyramine	Phenolic	No	No	No	Ö
N-cis-Feruloyltyramine	Phenolic	No	No	No	Ö

Several selected compounds have been reported to have promising anticancer activity. For instance, apigenin has anti-proliferative activity towards gastric cancer cells by inducing apoptosis [18]. In other report, apigenin also reported can inhibited ER-positive breast cancer proliferation through Akt/FOXM1 signaling [19]. On the other hand, b-sitosterol and b-stigmasterol exhibit cytotoxicity against cancer [20], [21]. The application of the network pharmacology approach is essential for systematically exploring the interactions between these compounds and their molecular targets. Therefore, a holistic understanding of their synergistic effect and the biological pathway involved in cancer suppression is necessary.

Protein-related Breast Cancer, Protein-target of Selected Compound, and Protein-Protein Interaction (PPI) Network

Protein-related breast cancer was identified using two databases, resulting in 705 proteins from GeneCards and 656 proteins from OMIM. Based on this, 951 non-duplicate data protein-related breast cancer cases were obtained. Subsequently, protein targets of 10 selected compounds were predicted using the SwissTarget Prediction database, resulting in 453 non-redundant targets. Integration of these datasets revealed an overlap between protein-related breast cancer and protein targets, which were subsequently subjected to PPI network construction.

A total of 72 proteins overlapping between protein-related breast cancer and protein-target were imported to the STRING database to construct PPI networking. The PPI network constructed from 72 proteins revealed a highly interconnected network consisting of 72 nodes and 340 edges. This PPI network is available in **Figure 2**. The average node degree in this PPI network is 9.44, indicating that each protein is connected with nearly ten other proteins, suggesting a high level of functional interdependence. Topological analysis of the PPI network was performed to identify key hub proteins, and the results are available in **Table S1**. Among these 72 proteins, EGFR, AKT1, ESR1, PIK3RQ, JUN, and PIK3CA exhibit the highest centrality values, indicating their essential regulatory roles in this PPI network. EGFR and AKT1 showed the highest centrality (0.12-0.13), highest degree (27), and closeness (0.6 – 0.61). This result shows that these two proteins act as hub nodes, facilitating signal transduction between pathways. However, three proteins, namely SLC16A1, PRKCH, and CTRC, do not interact with other proteins.

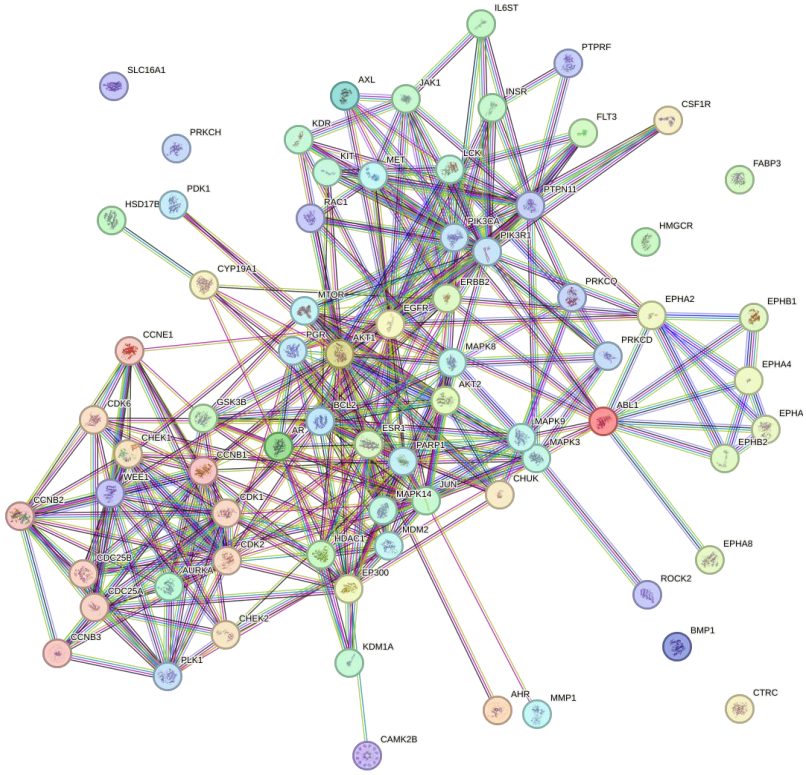


Figure 2. protein-protein interaction (PPI) network of overlapping between protein-related breast cancer and protein-target.

KEGG Pathway and Gene Ontology (GO) Analysis

The clustering process is an important step in identifying groups of proteins that have the same role. By performing clustering analysis of the PPI network, five clusters were obtained, as listed in **Table 3**. Notably, cluster 2 contains several key oncogenic hubs, namely EGFR, AKT1, ESR, JUN, and BCL2, and was therefore selected for downstream analysis. Furthermore, KEGG enrichment analysis (**Figure** 3) revealed that Cluster 2 was significantly associated with cancer-related pathways, particularly PI3K–AKT signaling, MAPK signaling, endocrine resistance, and breast cancer pathways. The enrichment of these pathways is mechanistically consistent with the central

roles of EGFR and AKT1 in promoting proliferation [22]. In breast cancer, aberrant activation of the EGFR/PI3K/AKT axis contributes to drives uncontrolled proliferation and apoptosis resistance, while ESR1-mediated enables hormone-dependent growth via estrogen-responsive gene transcription [22], [23], [24]. The presence of MAPK family members (MAPK3, MAPK8, MAPK14) further supports the involvement of mitogenic and stress-response signaling pathways that are frequently dysregulated in breast malignancies.

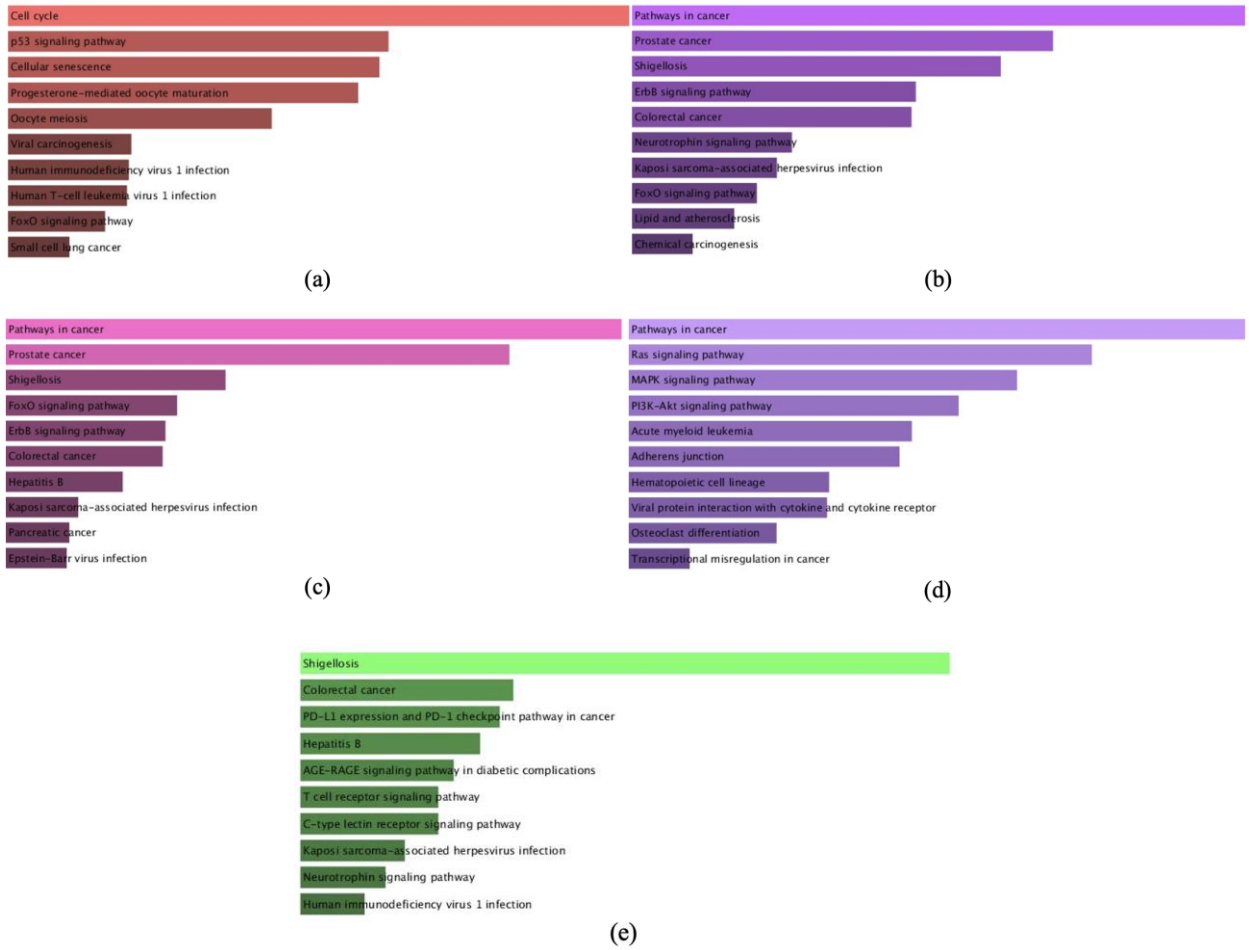


Figure 3. KEGG Pathway of each cluster (a) cluster 1 (b) cluster 2 (c) cluster 3 (d) cluster 4 and (e) cluster 5.

Table 3. Clustering analysis of 72 proteins on PPI network

Cluster	Representative protein
Cluster 1	CDK2, CCNB1, CDK1, CDC25A, WEE1, CHEK1, CCNB2, PLK1, CCNE1, AURKA, CDC25B, CDK6, CHEK2
Cluster 2	EGFR, AKT1, ESR1, JUN, BCL2, EP300, PARP1, MDM2, MTOR, PGR, MAPK8, MAPK14, AR, AKT2, MAPK3 , GSK3B, HDAC1, MAPK9, CHUK, KDM1A, PDK1, CAMK2B, AHR
Cluster 3	EGFR, AKT1, ESR1, JUN, BCL2, CDK2, ERBB2, EP300, PARP1, MDM2, MTOR, PGR, MAPK8, MAPK14, AR, AKT2, MAPK3, GSK3B, HDAC1, MAPK9, CHUK, MMP1
Cluster 4	AXL, RAC1, FLT3, IL6ST, CSF1R, PTPRF
Cluster 5	EGFR, AKT1, ESR1, JUN, BCL2, EP300, PARP1, MTOR, PGR, MAPK8, MAPK14, AKT2, MAPK3, MAPK9, CHUK, PRKCD, PRKCQ

Gene ontology (GO) analysis was performed to determine the biological role of proteins in cluster 2. The results of the analysis are available in **Figure 4.** GO analysis is divided into three categories, namely biological process (BP), molecular function (MF), and cellular component (CC). BP explains the role that proteins (or genes) play in biological processes such as apoptosis, growth, cell division, or signal transduction. CC indicates the specific location of protein (or genes) at the cellular level. Furthermore, MF describes the molecular activity of proteins (or genes) [25-26]. Understanding these three types of GO will provide a holistic understanding of how compounds can affect biological activity at the molecular and cellular levels.

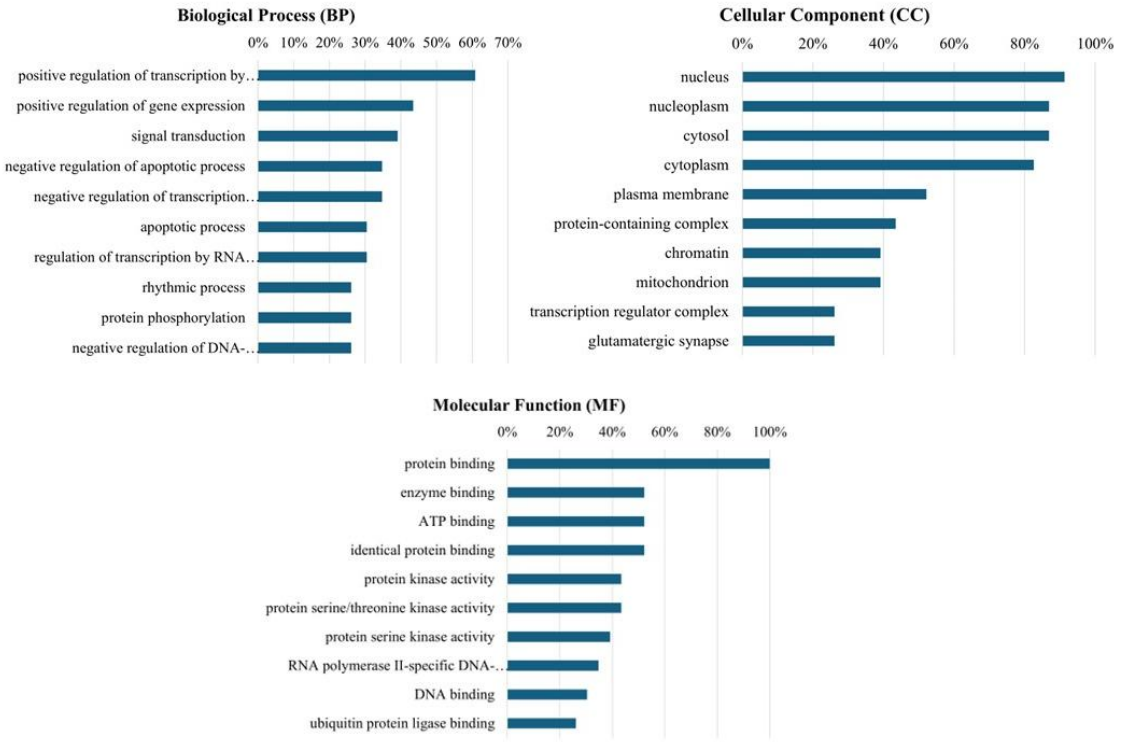


Figure 4. Gene ontology (GO) analysis consists of molecular function (MF), biological process (BP), and cellular component (CC)

GO analysis of cluster 2 further supported the explanation of KEGG enrichment. In the Biological Process category, enrichment of transcriptional regulation signal transduction terms suggests that the identified targets are primarily involved in coordinating gene expression and intracellular signaling networks critical for tumor growth and survival. Rather than representing general cellular processes, these enrichment terms reflect oncogenic mechanisms driven by kinase-mediated phosphorylation and transcription factor activation. In the Cellular Component category, the predominance of nuclear and cytoplasmic localization is consistent with the dual functional roles of these proteins in cytoplasmic signal propagation and nuclear transcriptional regulation. Molecular Function enrichment highlighted protein binding, enzyme binding, kinase activity, and ubiquitin ligase interactions, suggesting that post-translational regulatory mechanisms, such as phosphorylation and ubiquitination, may represent key targets for therapeutic intervention. In the Biological Process category, enriched terms were

predominantly associated with transcriptional regulation and intracellular signaling. Rather than representing isolated functional annotations, these processes collectively indicate that Cluster 2 proteins are centrally involved in coordinating kinase-driven signaling cascades and transcriptional programs that regulate tumor cell proliferation and survival. Such mechanisms are consistent with aberrant activation of EGFR–AKT–MAPK signaling networks in breast cancer, suggesting that the identified targets may influence oncogenic signal amplification and downstream gene expression.

Moreover, in MF, most of the proteins are in terms of protein binding and enzyme binding. This result suggests that targets possess strong interactive and catalytic roles. The presence of protein kinase activity and ubiquitin protein ligase binding indicates that phosphorylation and protein degradation pathways may be influenced, both of which play crucial roles in maintaining cellular homeostasis and regulating tumour cell survival. Collectively, the KEGG and GO analyses suggest that the selected compounds may exert anti-breast cancer effects by modulating central oncogenic hubs within the EGFR/AKT/MAPK signaling network, thereby interfering with proliferative, survival, and hormone-regulated pathways

Compound-protein-pathway Network.

To reveal the molecular mechanism among selected compounds, target proteins, and enriched pathways, a network of compound-protein-pathway was constructed, and the results are available in Figure 5. The topological analysis results exhibit 43 nodes and 185 edges, indicating a high degree of interaction among compounds and proteins associated with cancer pathways. The degree value represents the number of direct connections that a node has with other nodes in the network, indicating its level of connectivity and potential biological influence.

In this network, AKT1 and MAPK3 have the highest degree (14), followed by AKT2 and EGFR (12). These proteins act as a hub node, suggesting their central regulatory roles in mediating multiple signalling cascades associated with breast cancer progression. The high connectivity of AKT1 and MAPK3 implies that they act as key integrators of various upstream signals, including those derived from natural bioactive compounds, and may represent potential therapeutic targets. On the other hand, cycloeucalenone and apigenin exhibit the highest degree among other selected compounds. This result indicates these compounds have multiple interactions with other proteins. Especially, apigenin has been reported to have broad activity as an anticancer through broad modulation, such as PI3K-Akt, MAPK, and p53 signaling pathways. This suggests that apigenin could function as a multi-target modulator capable of influencing various molecular mechanisms in breast cancer. Not only apigenin, but cycloeucalenone has also been identified to have an interaction with some hub nodes in the network and has been reported to have antioxidant activity [27]. This indicates potential relations between cancer regulation and antioxidant activity, such as by reducing oxidative stress and modulating pathways through MAPK or AKT [28-29].

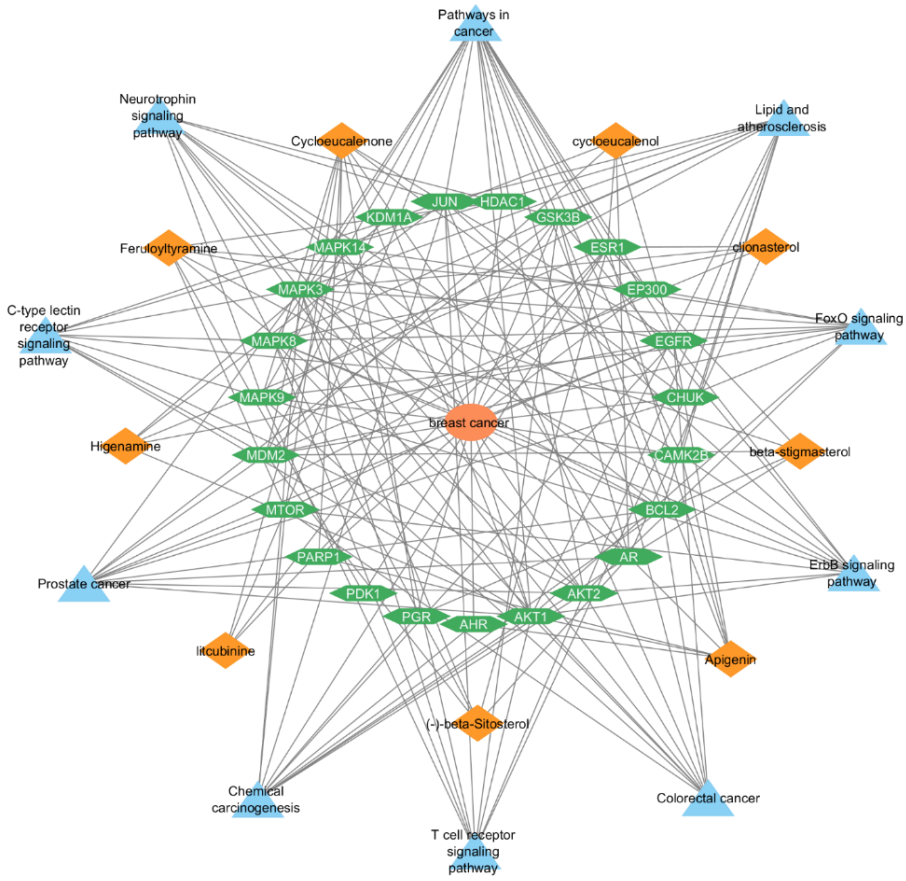


Figure 5. compound-protein-pathway network.

Molecular Docking of Selected Compound and Protein

The compound and protein with the highest degree were selected for further analysis in molecular docking. Two compounds and three proteins were chosen, indicating their strong interaction with multiple protein targets and play a major role in the compound-target-pathway network. In this study, docking was performed for apigenin and cycloeucalenone compounds with MAPK3, AKT1, and EGFR proteins because each had the highest degree among others in the same category. The crystal structures of MAPK3, AKT1, and EGFR were obtained from the protein data bank (PDB) with PDB IDs 4QTB, 4GV1, and 1M17. The molecular docking validation methods was conducted by re-docking native ligand from each structure. The root mean square deviation (RMSD) value indicates the deviation of the binding pose between the original ligand and the redocked ligand. The method used is valid if it has an RMSD value of less than 2 Å. The results of re-docking each ligand to the corresponding protein have a value of less than 2 Å, so the method used is valid. The result binding energy is summarized in Table 4, while the corresponding 2D interaction and binding site are available in Figure 5 and Table 5.

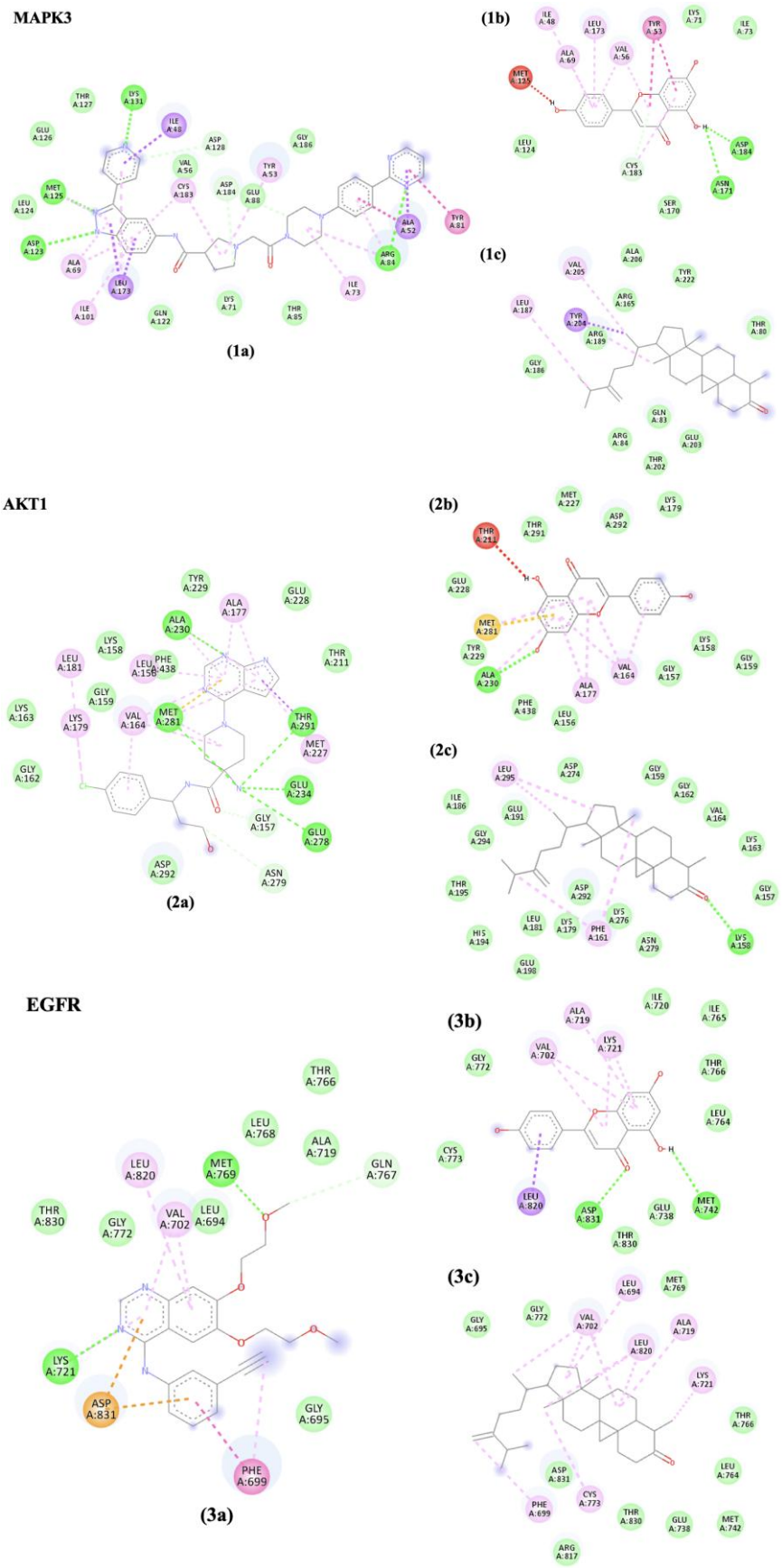


Figure 5. 2D interaction between chosen protein and compound. (1a) native ligand-MAPK3; (1b) apigenin-MAPK3; (1c) cycloeucalenone-MAPK3; (2a) native ligand-AKT1; (2b) apigenin-AKT1; (2c) cycloeucalenone-AKT1; (3a) native ligand-EGFR; (3b) apigenin-EGFR; (3c) cycloeucalenone-EGFR.

Among the tested results, cycloeucalenone exhibits the most stable binding with AKT1 and EGFR compared to apigenin and the native ligand. On the other hand, apigenin is more stable than cycloeucalenone, but less stable compared to the native ligand. 2D-structural analysis of the binding modes shows cycloeucalenone reproduces key interactions observed in the native ligand-EGFR complex. Although cycloeucalenone does not form classical hydrogen bonds with EGFR in the docked pose, it displays a more favourable docking energy than apigenin. Structural inspection shows cycloeucalenone occupies the hydrophobic region of the ATP-binding pocket more extensively, forming multiple van-der-Waals and hydrophobic contacts with residues such as Phe699, Val702, Leu820, and Met769. In the AKT1 complexes, both compounds occupied the ATP-binding pocket. Apigenin interacted with residues such as Met281 and Ala230, whereas cycloeucalenone showed hydrogen-bonding interactions only with Lys158. Although cycloeucalenone forms fewer hydrogen bonds, it has a more negative binding energy than apigenin. This is possible because cycloeucalenone interacts with more amino acid residues in the binding pocket than apigenin.

In contrast, in MAPK interaction, apigenin has a more negative binding energy than cycloeucalenone. The higher binding stability of apigenin can be explained by its ability to form multiple hydrogen bonds with key amino acid residues, such as Asp184 and Asn171, which play crucial roles in substrate recognition and catalytic function. Hydrogen bonds are essential contributors to the specificity of ligand–protein interactions, enhancing both orientation and binding strength. Furthermore, apigenin also establishes extensive hydrophobic contacts with residues located in the MAPK3 binding pocket, such as Val56, Leu173, and Ile74, which further stabilize the complex through van der Waals forces. More interaction forms in the hydrophobic interaction between apigenin and MAPK3, which supports the formation of more stable interactions in the MAPK3 binding pocket compared to cycloeucalenone.

Table 4. The binding energy between the protein and the compound based on molecular docking

Protein	Binding Energy			RMSD of re-docking results (Å)
	Native ligand*	Apigenin	Cycloeucalenone	
MAPK3	-14.1	-9.1	-7.4	-0.359
AKT1	-8.8	-7.9	-9.8	-0.205
EGFR	-7.3	-8.9	-9.6	-1.210

**different native ligand is used depend on the protein*

Table 5. The binding site interaction between the selected protein and compounds

Ligand	Binding Sites	
	Hydrogen Bonds	Hydrophobic interaction
1a	Lys131, Met125, Asp123, Arg84	Gl126, Thr127, Val56, Cys183, Asp128, Asp184, Glu88, Tyr53, Gly186, Leu124, Ala69, Ile101, Leu173, Gln122, Lys71, Thr85, Ile73, Arg84, Ala52, Tyr81
1b	Asp184, Asn171	Ile48, Ala69, Leu173, Val56, Tyr53, Lys71, Ile73, Met125, Leu124, Cys183, Ser170

Ligand	Binding Sites	
	Hydrogen Bonds	Hydrophobic interaction
1c	-	Leu187, Tyr204, Arg189, Arg165, Val206, Tyr222, Thr80, Glu203, Gln83, Arg84
2a	Met281, Ala230, Thr291, Glu234, Glu278	Lys163, Lue181, Lys179 Gly159, Lys158, Leu156, Val164, Phe438, Tyr229, Ala177, Glu228, Thr211, Met227, Gly157, Asn279, Asp292, Gly162
2b	Met281, Ala230	Glu228, Thr211, Thr291, Met227, Asp282, Lys179, Tyr229, Phe438, Leu156, Ala177, Val164, Gly157, Lys158, Gly159,
2c	Lys158	Leu295, Asp274, Gly159, Gly162, Val164, Lys163m Gly157, Asn279, Lys276, Asp292, Phe161, Lys179, Leu181, Glu198, His194, Thr195 Gly294, Glu191, Ile186ß
3a	Lys721, Met769	Thr830, Gly772, Leu820, Val702, Leu694, Leu768, Thr766, Ala719, Gln767, Gly695, Phe699, Asp831
3b	Asp831, Met742	Gly772, Val702, Ala719, Lys721, Ile720, Ile765, Thr766, Leu764, Glu738, Thr830, Leu820, Cys773
3c	-	Gly695, Gly772, Val702, Leu694, Leu820, Met769, Ala719, Lys721, Thr766, Leu764, Met742, Glu738, Thr830, Cys773, Asp831, Phe699, Arg817

Conclusions

This study aimed to elucidate the potential anticancer properties of *T.crispa* components through an integrative analysis between network pharmacology and molecular docking approach. The compound collection and selection process identified 9 bioactive compounds that satisfied the ADMET and Lipinski rule of five. Protein-related breast cancer analysis identified 72 proteins overlapping protein targets between *T. crispa* bioactive compounds and breast cancer-related proteins, forming a highly connected protein–protein interaction (PPI) network. Cluster analysis revealed that cluster 2 was significantly associated with cancer-related pathways, including AKT1, MAPK, and EGFR signaling, indicating a strong relationship between these targets and breast cancer development. Gene Ontology (GO) enrichment further demonstrated that most proteins were localized in the nucleus and were involved in transcriptional regulation, protein binding, and kinase activity, suggesting their functional importance in cellular proliferation and survival processes. Among the screened compounds, apigenin and cycloeucalenone exhibited the highest degree values based on the compound-protein-pathway network, highlighting their central roles in the compound–target network. Molecular docking confirmed that cycloeucalenone displayed a more stable interaction between EGFR and AKT1 than apigenin, primarily through hydrophobic interactions, whereas apigenin formed multiple hydrogen bonds with MAPK3, resulting in a more stable complex within the binding pocket. These findings collectively indicate that *T. crispa* may have potential anticancer properties, as predicted by computational analyses, possibly through multi-target modulation of interconnected signaling pathways. However, further in vitro and in vivo studies are required to experimentally validate these predicted mechanisms.

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Author Contributions

Conceptualization and study design were carried out by all authors. Data collection and curation were performed by Novelia Andini and elon Jipasca. Network pharmacology analysis was conducted by the Septaria Yolan Kalilinggi and Mirnawati Dewi. The molecular docking, was performed by Mokhamat Ariefin. Data interpretation and validation were contributed by all authors. The manuscript was written by the Mirnawati Dewi with contributions and critical revisions from all co-authors. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the selection of the research topic; in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

[1]. Andi Herawati, Syamsu Rijal, Arsal ASF, Reeny Purnamasari, Abdi DA, Syarifuddin Wahid. Karakteristik Kanker Payudara. *Fakumi Medical Journal: Jurnal Mahasiswa Kedokteran*. 2021,1(1):44–53. DOI: 10.33096/fmj.v1i1.76

[2]. Ozmen V. Breast cancer in the world and Turkey. *Journal of Breast Health*. 2008;4(2):6–12.

[3]. Yun D, Yoon SY, Park SJ, Park YJ. The Anticancer Effect of Natural Plant Alkaloid Isoquinolines. *International Journal of Molecular Sciences*. 2021,22(4):1653. DOI: 10.3390/ijms22041653

[4]. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. *Nutrients*. 2020;12(2):457. DOI:10.3390/nu12020457

[5]. Othman A, Mukhtar NJ, Ismail NS, Chang SK, Alam S., Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants. *International Food Research Journal*. 2014.21(2):759-766.

[6]. Warsinah W, Baroroh HN, Harwoko H. Phytochemical Analysis and Antioxidant Activity of Brotowali (*Tinospora crispa* L. Mier) Stem. *Molekul*. 2020. 27;15(2):73. DOI:10.20884/1.jm.2020.15.2.533

[7]. Yusoff M, Hamid H, Houghton P. Anticholinesterase Inhibitory Activity of Quaternary Alkaloids from *Tinospora crispa*. *Molecules*. 2014, 20;19(1):1201–11. DOI:10.3390/molecules19011201

[8]. Martani NS, Fatmaria F. Pemanfaatan Penawar Sampai (*Tinospora crispa* L.) sebagai Tanaman Obat Tradisional oleh Suku Dayak Ngaju. *Jurnal Pharmascience*. 2020. 31;7(2):156. DOI: 10.20527/jps.v7i2.8799

[9]. Hopkins AL. Network pharmacology. *Nature Biotechnology*. 2007, 25(10):1110–1. DOI: 10.1038/nbt1007-1110

[10]. Noor F, Tahir ul Qamar M, Ashfaq UA, Albutti A, Alwashmi ASS, Aljasir MA. Network Pharmacology Approach for Medicinal Plants: Review and Assessment. *Pharmaceuticals*. 2022;15(5):572. DOI:10.3390/ph15050572

[11]. Jiao X, Jin X, Ma Y, Yang Y, Li J, Liang L, et al. A comprehensive application: Molecular docking and network pharmacology for the prediction of bioactive constituents and elucidation of mechanisms of action in component-based Chinese medicine. *Computational Biology and Chemistry*. 2021. 90:107402. DOI:10.1016/j.compbiolchem.2020.107402

[12]. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. *Biophysical Review*. 2017; 91–102. DOI: 10.1007/s12551-016-0247-1

[13]. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. *International Journal of Molecular Sciences*.

2019, 4;20(18):4331. DOI:10.3390/ijms20184331

[14]. Puthanveedu V, Muraleedharan K. Phytochemicals as potential inhibitors for COVID-19 revealed by molecular docking, molecular dynamic simulation, and DFT studies. *Structural Chemistry*. 2022. 13;33(5):1423–43. DOI:10.1007/s11224-022-01982-4

[15]. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Journal of Chemical Information and Modeling.* 2021. 23;61(8):3891–8. DOI: 10.1021/acs.jcim.1c00203

[16]. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*. 2009;31(2):455-461.DOI:10.1002/jcc.21334

[17]. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technology*. 2004,1(4):337–41. DOI:10.1016/j.ddtec.2004.11.007

[18]. Pratas A, Malhão B, Palma R, Mendonça P, Cervantes R, Marques-Ramos A. Effects of apigenin on gastric cancer cells. *Biomedicine & Pharmacotherapy.* 2024.172:116251. DOI:10.1016/j.biopha.2024.116251

[19]. Pham TH, Page Y Le, Percevault F, Ferrière F, Flouriot G, Pakdel F. Apigenin, a Partial Antagonist of the Estrogen Receptor (ER), Inhibits ER-Positive Breast Cancer Cell Proliferation through Akt/FOXM1 Signaling. *International Journal of Molecular Sciences*. 2021,22(1):470. DOI:10.3390/ijms22010470

[20]. Zhang Y, Yang C, Guo J, Yan L, Li Y, Zhang Y. Anticancer Potential of β-Sitosterol: A Comprehensive Exploration of Mechanisms and Therapeutic Prospects. *The American Journal of Chinese Medicine*. 2025;53(04):1241–63. DOI: 10.1142/S0192415X2550048X

[21]. Farshori NN, Al-Oqail MM, Al-Sheddi ES, Al-Massarani SM, Alam P, Siddiqui MA, et al. HPTLC estimation and anticancer potential of Aloe perryi petroleum ether extract (APPeE): A mechanistic study on human breast cancer cells (MDA-MB-231). *Journal of King Saud University-Science*. 2022. 34(4):101968. DOI: 10.1016/j.jksus.2022.101968

[22]. Cairns J, Fridley BL, Jenkins GD, Zhuang Y, Yu J, Wang L. Differential roles of ERRFI1 in EGFR and AKT pathway regulation affect cancer proliferation. *EMBO Reports*. 2018. 15;19(3). DOI:10.15252/embr.201744767

[23]. Yang S, Manna C, Manna PR. Harnessing the Role of ESR1 in Breast Cancer: Correlation with microRNA, lncRNA, and Methylation. *International Journal of Molecular Sciences*. 2025. 27;26(7):3101. DOI:10.3390/ijms26073101

[24]. Kaboli PJ, Imani S, Jomhori M, Ling KH. Chemoresistance in breast cancer: PI3K/Akt pathway inhibitors vs the current chemotherapy. *American Journal of Cancer Research*. 2021;11(10):5155–83.

[25]. Smith B, Williams J, Schulze-Kremer S. The ontology of the gene ontology. *AMIA Annual Symposium Proceedings*. 2003;609–13. PMID: 14728245.

[26]. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. *Nature Genetics*. 2000, 25(1):25–9. DOI: 10.1038/75556

[27]. Ali IJ, Olofinsan KA, Ikeh GO, Okorie NH, Adonu CC, Omeh RC, et al. Anti-Inflammatory, Antioxidant, Molecular Docking and Pharmacokinetics Properties of Cycloeucalenone Isolated from *Musa paradisiaca* L. Fruit Peels. *Chemistry Biodiversity.* 2025. 23(1). DOI:10.1002/cbdv.202502342

[28]. Dong R, Wang J, Guan R, Sun J, Jin P, Shen J. Role of Oxidative Stress in the Occurrence, Development, and Treatment of Breast Cancer. *Antioxidants*. 2025, 17;14(1):104. DOI:10.3390/antiox14010104

[29]. Shiau JP, Chuang YT, Tang JY, Yang KH, Chang FR, Hou MF, et al. The Impact of Oxidative Stress and AKT Pathway on Cancer Cell Functions and Its Application to Natural Products. *Antioxidants*. 2022. 19;11(9):1845. DOI: 10.3390/antiox11091845