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Exploration of Multitarget Anti-Breast Cancer Potential of Selected Flavonoids Through Network Pharmacology and Molecular Docking

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Abstract

Breast cancer remains a leading cause of mortality among women worldwide, including in Indonesia. Although current therapies such as chemotherapy, radiotherapy, and hormone therapy have improved patient outcomes, their effectiveness is frequently limited by systemic toxicity and multidrug resistance. In this study, the therapeutic potential of six natural flavonoid compounds, selected based on their reported occurrence in *Annona muricata* and documented anticancer related activities, was evaluated using a computational biology approach. Network pharmacology analysis identified estrogen receptor (ER-α) (PDB ID: 3ERT), anti-apoptotic protein (BCL-2) (PDB ID: 6GL8), and DNA repair enzyme (PARP1) (PDB ID: 4R6E) as key molecular targets involved in breast cancer progression. The compounds were assessed through molecular docking, Lipinski's Rule of Five, and absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) predictions to identify promising candidates with favorable pharmacokinetic and safety profiles. Among the evaluated compounds, rutin and kaempferol exhibited the most favorable overall characteristics based on binding affinity and predicted toxicity. These findings provide preliminary computational evidence supporting the potential of these flavonoids as lead candidates for further development. Nevertheless, experimental validation through molecular dynamics and biological studies is required to confirm their therapeutic relevance.

Keywords: Breast cancer therapy, Cancer apoptotic pathway, Hormone receptor antagonists, In silico analysis, Natural compound screening

Eksplorasi Potensi Antikanker Payudara Multitarget dari Flavonoid Terpilih melalui Pendekatan Network Pharmacology dan Molecular Docking

Abstrak

Kanker payudara masih menjadi salah satu penyebab utama mortalitas pada perempuan di seluruh dunia termasuk di Indonesia. Meskipun terapi yang ada seperti kemoterapi, radioterapi, dan terapi hormon telah meningkatkan luaran klinis pasien, efektivitasnya sering kali dibatasi oleh toksisitas sistemik dan *multidrug resistance*. Dalam penelitian ini, potensi terapeutik enam senyawa flavonoid alami yang terdokumentasi dalam *Annona muricata* dan aktivitasnya sebagai antikaker, dievaluasi menggunakan pendekatan biologi komputasional. Analisis farmakologi jaringan mengidentifikasi reseptor estrogen ER-α) (PDB ID: 3ERT), protein antiapoptotik (BCL-2) (PDB ID: 6GL8), dan enzim perbaikan DNA (PARP1) (PDB ID: 4R6E) sebagai target molekuler utama yang terlibat dalam progresi kanker payudara. Senyawa-senyawa tersebut dianalisis melalui *molecular docking*, *Lipinski's Rule of Five*, serta prediksi absorpsi, distribusi, metabolisme, ekskresi, dan toksisitas (ADME-Tox) untuk mengidentifikasi kandidat yang menjanjikan dengan profil farmakokinetik dan keamanan yang baik. Di antara senyawa yang dievaluasi, rutin dan kaempferol menunjukkan karakteristik keseluruhan yang paling menguntungkan berdasarkan afinitas ikatan dan prediksi toksisitas. Temuan ini memberikan bukti komputasional awal yang mendukung potensi flavonoid tersebut sebagai kandidat utama untuk pengembangan lebih lanjut. Namun, validasi eksperimental melalui simulasi dinamika molekuler dan studi biologis tetap diperlukan untuk mengonfirmasi relevansi terapeutiknya.

Kata kunci: Analisis *in silico*, Antagonis reseptor hormon, Jalur apoptosis kanker, Skrining senyawa alami, Terapi kanker payudara

1. Introduction

Cancer is a disease characterized by uncontrolled cellular proliferation resulting from cellular evolutionary processes.[1]Based on GLOBOCAN data from 2022, Indonesia recorded over 408.661 new cancer cases and approximately 242.099 cancer-related deaths. Among these, breast cancer was the primary cause of cancer mortality in women (66.271 cases), representing a critical public health challenge that necessitates urgent therapeutic advancements.[2] Breast cancer is a malignant neoplasm originating from the mammary gland and represents a multifactorial disease arising from genetic, epigenetic, environmental, and lifestyle-related factors.[3] Its pathogenesis involves the dysregulation of complex biological networks that govern cell survival, proliferation, and metastasis.[4] Unlike diseases driven by a single genetic mutation, breast cancer progression is maintained by redundant signaling pathways and the aberrant activity of multiple regulatory proteins.[5] This systemic complexity often allows cancer cells to bypass the blockade of single-target therapeutics, leading to therapeutic failure.

Conventional breast cancer treatments, such as chemotherapy, radiotherapy, and hormone therapy have led to substantial improvements in disease management and patient survival, they remain far from ideal. These interventions are frequently associated with considerable limitations, including systemic toxicity, poor selective toward malignant cells, and the induction of multidrug resistance, which often culminates in therapeutic failure and disease recurrence.[6,7] Moreover, the cumulative burden of side effects from long-term treatment can severely impact patients' quality of life.[8] These challenges have shifted research attention toward natural bioactive compounds with multitarget actions and minimum toxicity. Flavonoids, a diverse group of polyphenolic compounds found abundantly in fruits, vegetables, and medicinal plants, have shown potential anticancer effects. These effects include the regulation of enzymes that neutralize reactive oxygen species (ROS), the ability to stop the cell cycle, the promotion of apoptosis and autophagy, as well as inhibition of tumor growth and metastasis.[9] Epidemiological evidence consistently demonstrates an inverse correlation between the dietary intake of flavonoid-rich foods and breast cancer incidence, suggesting a significant protective role for these metabolites.[10] Furthermore, this protective benefit extends into survivorship, higher flavonoid consumption has been associated with reduced cancer recurrence rates, particularly among overweight and obese patients where it significantly lowers the risk of relapse.[11] Their capacity to modulate multiple molecular pathways positions them as promising candidates for the development of complementary or alternative therapeutic strategies against cancer.

However, the 'multi-component, multi-target' nature of flavonoids presents a significant research challenge, as natural products typically act through the modulation of multiple targets rather than a single, highly specific one. Traditional pharmacological approaches, which adhere to a 'one drug, one target' reductionist paradigm, are often insufficient to elucidate the holistic mechanisms by which these compounds combat breast cancer. As a result, the specific key targets and the broader biological pathways responsible for the therapeutic effects of flavonoids remain largely unclear and continue to be explored. Identifying these critical molecular interactions is vital for transforming traditional knowledge into precision medicine.[12]

In silico studies, which leverage computational techniques to simulate and analyze biological processes and predict compound efficacy against various diseases, have become essential tools in modern drug discovery, offering promising methods to accelerate development and reduce costs by minimizing the need for extensive laboratory experiments.[13] These studies include methods such as network pharmacology and molecular docking. Network pharmacology is an interdisciplinary, systems-level approach that integrates pharmacology, systems biology, and computational network analysis to investigate how drugs act on multiple targets and pathways within biological

networks, rather than on a single target, in order to elucidate therapeutic mechanisms in complex disease.[14–16] Molecular docking is a computational technique designed to predict the binding affinity of ligands to receptor proteins. It has developed into a formidable tool for drug development, facilitating the identification of precise molecular targets to support rational drug design and the creation of disease-specific therapies.[17]

This study employs a comprehensive in silico approach to systematically uncover the therapeutic mechanisms of flavonoids in treating breast cancer. A robust 'compound-target-disease' network is constructed to identify key biological targets and signaling pathways involved in the disease. To validate these computational predictions, molecular docking is utilized to assess the interactions between flavonoids and their respective targets. Furthermore, the drug-likeness and pharmacokinetic profiles of these compounds are evaluated using Lipinski's Rule of Five and absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) analysis. These steps provide theoretical foundation for the potential development of flavonoid-based therapies aimed at improving breast cancer treatment outcomes.

2. Methods

2.1. Tools

All computational procedures were executed on a personal laptop with an AMD Ryzen 3 3250U processor with Radeon Graphics 2.60 GHz, 8.00 GB RAM, and Windows 64-bit operating system. The software and web tools utilized were BIOVIA Discovery Studio 2025, PubChem, Protein Data Bank, SwissADME, Chem3D Pro 12.0, AutoDock 4.2.6, PreADMET, SwissTargetPrediction, GeneCards, Cytoscape 3.10.4, and ShinyGO 0.85.1. The core target proteins employed in this study included the ER-α receptor (PDB ID: 3ERT), BCL-2 (PDB ID: 6GL8) and PARP1 (PDB ID: 4R6E), each accompanied by their native ligands retrieved from the Protein Data Bank (PDB). Test flavonoid compounds sourced from PubChem and optimized using Chem3D Pro 12.0 software with MM2 geometry optimization, as depicted in Figure 1.

2.2. Procedures

2.2.1. Lipinski's rule of 5 predictions

Lipinski Rule of 5 (RO5) predictions were generated by obtaining the SMILES format representations of each test compound from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and processing their two-dimensional structural

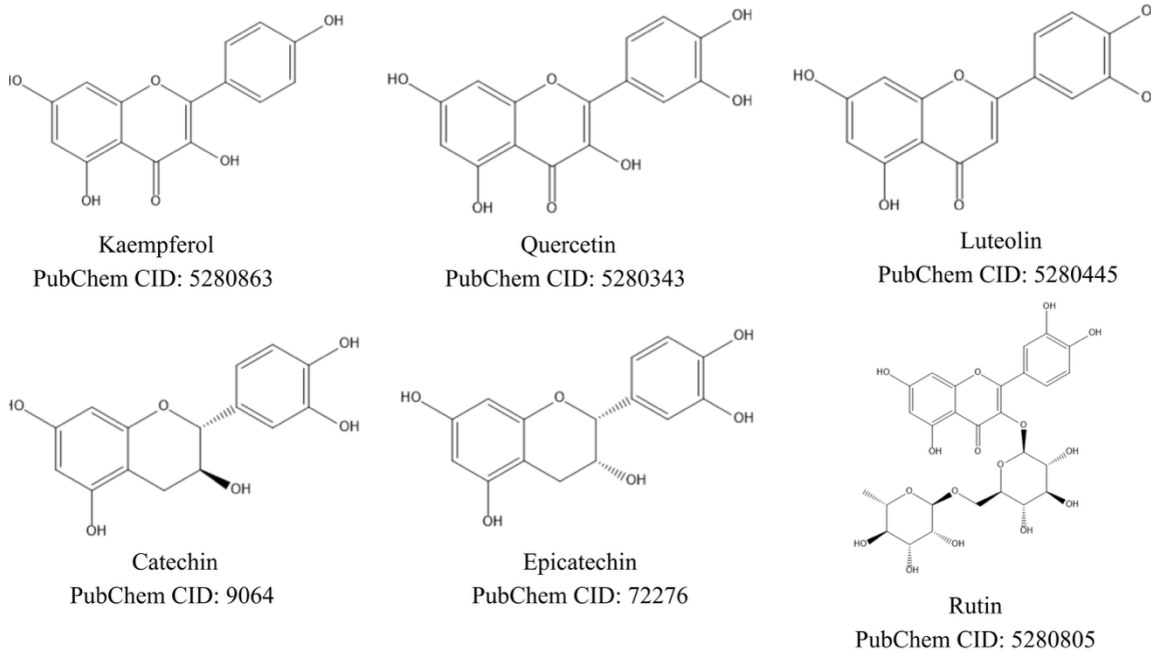

Figure 1. Chemical structure of flavonoids used as test ligands.

forms. The compounds were subsequently evaluated using SwissADME (http://www.swissadme.ch/) in order to determine the drug-likeness. The drug-likeness parameters including molecular weight, log P, hydrogen bond donors, and hydrogen bond acceptors were assessed in accordance with Lipinski's RO5 criteria.

2.2.2. ADME-Tox predictions

Pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADME- Tox) predictions were carried out by retrieving the test compounds from PubChem and subjecting them to analysis using PreADMET (https://preadmet.webservice.bmdrc.org/). The pharmacokinetics parameters evaluated included Human Intestinal Absorption (HIA), distribution to blood-brain barrier (BBB), plasma protein binding (PPB), and permeability towards Caco-2 cells were evaluated. Additionally, toxicity indicators such as rodent carcinogenicity and Ames test were also assessed.

2.2.3. Prediction of Compound Targets

The evaluated flavonoid compounds used in this study were selected based on literature reports indicating their presence in *Annona muricata* and their known antioxidant, anti-proliverative, and apoptosis modulating activities, and which structural data were available in PubChem (https://pubchem.ncbi.nlm.nih.gov/), as seen in Figure 1. The potential targets of the flavonoids were identified using SwissTargetPrediction (STP) (https://www.swisstargetprediction.ch/) and Similarity Ensemble Approach (SEA) (https://sea.bkslab.org/) under the Homo sapiens settings.

2.2.4. Identification of Related Targets for Breast Cancer

The related targets for inflammation were identified using the GeneCards platform (https://www.genecards.org/) with "breast cancer" AND "anti-breast cancer" as the key words. Moreover, the species was altered to Homo sapiens and the disease relevance score threshold ≥10 was employed. DisGeNET (https://disgenet.com/) platform was also used to identify the breast cancer related targets with gene-disease association score of ≥0.3. The overlapping targets between the flavonoids were identified and visualized by Venny 2.1.0 diagram webtools (https://bioinfogp.cnb.csic.es/tools/venny/).

2.2.5. Construction of Compound-Target Network

Following prior overlapping targets analysis, the flavonoids and the predicted compound targets were matched with the breast cancer targets in order to obtain the compound target network. The interaction network between the active compounds and inflammation-related targets was subsequently established and visualized using Cytoscape 3.10.3 (https://cytoscape.org/).

2.2.6. Construction of Protein-Protein Interaction (PPI) Network

The overlapping targets were imported into STRING (https://string-db.org/) with a medium confidence level of 0.700 in order to analyze the PPI with the organism parameter set to Homo sapiens setting. The resulting interaction data were visualized and analyzed using Cytoscape 3.10.3. Topological analysis was performed to assess the significance of each node, where the degree value represents the number of direct connections a node exhibited within the network. Furthermore, topological analysis serves to determine whether a target protein is an important basis for key targets. In this study, the top three nodes with highest degree values were identified as "core targets", which indicates their potential important roles in the network.

2.2.7. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Investigation

GO and KEGG pathway enrichment investigation were performed using the ShinyGO 0.85.1 platform (https://bioinformatics.sdstate.edu/go/). A false discovery rate (FDR) threshold of 0.05 for both GO and KEGG analysis. The top 20 enriched terms were subsequently selected for visualization.

2.2.8. Molecular docking simulation

Geometry optimization of the ligands was performed prior to molecular docking using molecular mechanics (MM2) in Chem3D Pro 12.0. Each ligand was constructed and subjected to energy minimization to minimum RMS gradient of 0.010 using MM2 calculations. Molecular docking validation was initiated by re-docking the native ligands into the core target receptors using AutoDock 4.2.6. The grid box size and coordinates were optimized to achieve a reference RMSD value of ≤ 2 Å and a negative binding energy, with a Lamarckian Genetic Algorithm of 100. The grid box parameters for molecular docking were configured as follows: for the ER-α receptor (PDB ID: 3ERT), the grid box dimensions were set to 40 × 40 × 40 with center coordinates at x = 30.01, y = -1.913, z = 24.207; for the BCL-2 receptor (PDB ID: 6GL8), the grid box was also 40 × 40 × 40 with center coordinates at x = 17.189, y = 2.647, z = 15.648; and for PARP (PDB ID: 4R6E), the grid box dimensions were maintained at 40 × 40 × 40 with coordinates centered at x = -69.254, y = 23.260, z = 26.746. Afterwards, the molecular docking simulations were carried out on the prepared ER-α receptor (PDB ID: 3ERT), BCL-2 (PDB ID: 6GL8) and PARP1 (PDB ID: 4R6E), and the flavonoid test ligands. Receptor preparations were performed using BIOVIA Discovery Studio 2025 by removing the water molecules and separating the native ligand from the receptor. Kollman charges and hydrogen bonds were added to the receptor's polar regions. During the AutoDock ligand preparation process, nonpolar hydrogens were merged and hydrogen bonds and Gasteiger charges were added. Molecular docking used a Genetic Algorithm Lamarckian value of 100 and proceeded in a manner similar to that of the validation phase. AutoDock was used to examine the data and calculate the inhibition constants and binding energies. The BIOVIA Discovery Studio was used to visualize ligand-receptor interactions in both two and three dimensions.

3. Result

3.1. Lipinski's rule of five prediction

Lipinski's Rule of Five (RO5) analysis showed that all evaluated flavonoid compounds fulfilled the RO5 parameters, indicating favorable oral drug-likeness profiles. Rutin was the only compound that exhibited multiple violations, deviating from three criterias, suggesting lower predicted oral bioavailability compared to the other flavonoid (Table 1).

3.2. ADME-Tox prediction

The ADME-Tox prediction analysis was

Table 1. Lipinski's RO5 prediction results of flavonoid compounds

Compounds	Molecular weight (Da)	Log P	Hydrogen bond donor	Hydrogen bond acceptor	Lipinski violations
Quercetin (PubChem CID: 5280343)	302.23	1.988	5	7	0
Kaempferol (PubChem CID: 5280863)	286.24	2.282	4	6	0
Catechin (PubChem CID: 9064)	290.27	1.546	5	6	0
Epicatechin PubChem (CID: 72276)	290.27	1.546	5	6	0
Luteolin PubChem (CID: 5280445)	286.24	2.282	4	6	0
Rutin PubChem (CID: 5280805)	610.5	-1.687	10	16	3

performed to assess the pharmacokinetic properties and potential toxicity of the evaluated flavonoid compounds. Key ADME parameters, including absorption, distribution, metabolism, and excretion, were evaluated to determine their suitability for systemic administration and therapeutic use18 Additionally, toxicity profiles were generated to identify any safety concerns that might limit clinical applicability.[19] The summarized ADME-Tox results are presented in Table 2. The HIA analysis showed moderate to high gastrointestinal absorption for most compounds, with kaempferol and luteolin showing the highest predicted values (79.439% and 79.427%), indicating favorable oral bioavailability. Conversely, rutin demonstrated extremely poor absorption (2.861%), suggesting limited effectiveness when administered orally. Caco-2 permeability also varied, with kaempferol showing the highest permeability (9.577 nm sec-1), while catechin and epicatechin demonstrated minimal permeability (0.657 nm sec-1), reflecting potentially limited intestinal transport. Most evaluated flavonoids displayed high PPB value (> 89%), with catechin and epicatechin reaching 100%. In contrast, rutin showed a relatively low PPB value (43.9%), indicating a larger free fraction in circulation. This increased free fraction may enhance its immediate therapeutic potential. Additionally, all of the flavonoid compounds, including rutin, exhibit low BBB penetration, which may be advantageous by minimizing central nervous system (CNS) exposure. Rutin had the lowest predicted BBB value (0.029), while catechin and epicatechin had slightly higher values (0.395), yet all remained below the threshold typically associated with effective BBB penetration. Regarding toxicity, mutagenicity was predicted for all compounds except rutin, indicating potential genetic risk for most flavonoids. Carcinogenicity in rats was predicted for quercetin and kaempferol, while all compounds were classified as non-carcinogenic in mice.

3.3. Overlap Analysis Between Flavonoid Targets and Breast Cancer-Related Targets

Through the database screening, 898 targets associated with breast cancer were retrieved. Following the intersection of 142 compound targets and breast cancer-related targets, a total of 27 overlapping targets were identified, as depicted in Figure 2. This intersection represents potential therapeutic targets through which flavonoids may exert anti-breast cancer effects. Therefore, these 27 proteins are the most pertinent candidates for additional investigation since it shows that only a subset of flavonoid-related proteins are directly involved in breast cancer pathways. In order to further elucidate the potential mechanisms of flavonoids as an anti- breast cancer agent, a compound-target network was constructed using Cytoscape 3.10.3, as illustrated in Figure 3a. In this network, the orange nodes represent the flavonoid compounds, meanwhile the blue nodes represent target proteins that are associated with breast cancer. Furthermore, the connections (edges) show the predicted interactions between the flavonoids and their molecular

Table 2. ADMET results of the evaluated flavonoid compounds

Compounds	HIA (%)	Caco-2 (nm sec-1)	PPB (%)	BBB	Mutagen	Carcinogen	
						Mouse	Rat
Quercetin	63.485	3.413	93.236	0.173	Mutagen	7	0
Kaempferol	79.439	9.577	89.608	0.286	Mutagen	6	0
Catechin	66.708	0.657	100.000	0.395	Mutagen	6	0
Epicatechin	66.708	0.657	100.000	0.395	Mutagen	6	0
Luteolin	79.427	4.540	99.717	0.368	Mutagen	6	0
Rutin	2.861	7.913	43.898	0.029	Non-Mutagen	16	3

Abbreviations: HIA, Human intestinal absorption; PPB, Protein plasma binding; BBB, blood-brain barrier

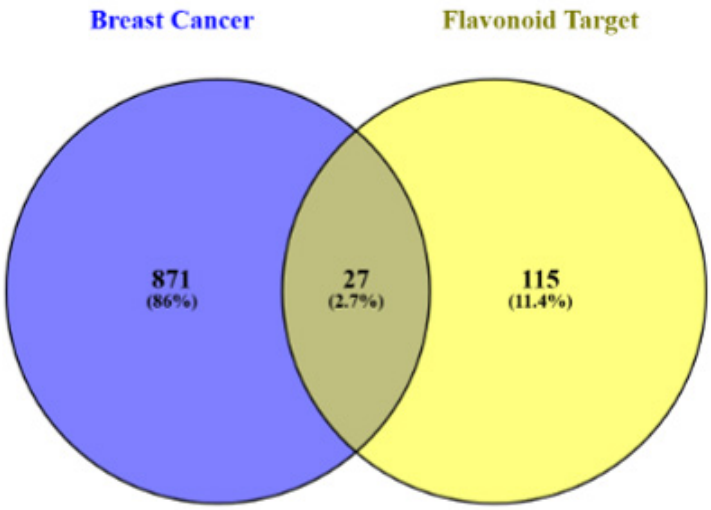

Figure 2. Venn diagram of the overlapping targets in flavonoid compounds and breast cancer

targets. These targets are involved in key biological processes in breast cancer including cell cycle regulation, apoptosis, and hormone signalling, which suggest that flavonoids may inhibit breast cancer progression through multiple pathways.

3.4. PPI Network

The PPI network of the 27 overlapping targets were constructed as seen in Figure 3b. The node color intensity represents the degree value, with darker shades demonstrating higher connectivity. Among these PPI network, ER-α, BCL-2, and PARP1 exhibited the highest degree values, which implied their dominant role as key regulatory target within the network. Therefore, these findings demonstrated that the biological activities of the analyzed flavonoids may be closely associated with the modulation of these core targets, which are recognized for their important involvement in breast cancer signaling pathways.

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

GO and KEGG analysis were performed in order to determine the biological relevance of flavonoid against breast cancer. Figure 4 displayed the most enriched GO terms for biological process (BP), cellular component (CC), and molecular function (MF). Furthermore, the top KEGG pathways were also shown in Figure 4. According to the GO-

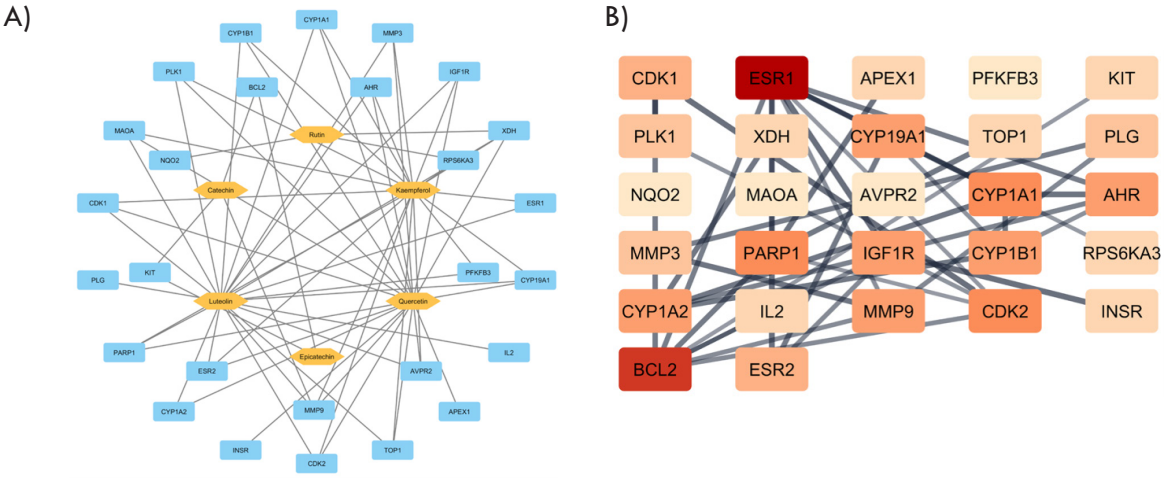

Figure 3. (*A*) Compound–target network of flavonoid compounds and breast cancer; (**B**) PPI network of 26 targets of flavonoid compounds against breast cancer.

BP, the targets were predominantly involved in various processes such as peptidyl- serine phosphorylation, rhythmic process, and peptidyl-amino acid modification. Within the GO-CC findings, the enriched terms demonstrated that the targets were mainly localized in the insulin receptor complex, cyclin a2-cdk2 complex, and cyclin e1–cdk2 complex. GO-MF enrichment further unveiled the strong correlation between the insulin receptor activity, nuclear estrogen receptor activity, and hydroperoxy icosatetraenoate dehydrase activity. In consistent with the GO analysis, KEGG pathway enrichment demonstrated that the targets were highly associated with multiple cancer-related pathways, with notable enrichment in the breast cancer pathway itself. Furthermore, the KEGG enrichment revealed strong associations with ovarian steroidogenesis, steroid hormone biosynthesis, and estrogen signaling pathway which highlighted the significant role of hormonal regulation in breast cancer development and progression. Additionally, enrichment of another important oncogenic pathway including PI3K- Akt, MAPK, FoxO, and HIF-1 signaling pathway.

3.6. Molecular docking simulation

Prior to conducting molecular docking simulations on the evaluated flavonoid compounds, the docking protocol was first validated to ensure accurate identification of the receptor active sites and appropriate grid box parameters. Redocking of the native ligands yielded well-aligned superimpositions with a root mean square deviation (RMSD) values of 1.27 Å (ER-α), 0.77 Å (BCL-2), and 0.48 Å for (PARP1). RMSD is a critical parameter of evaluating the precision of docking predictions, with values of ≤ 2 Å generally reflecting reliable binding pose accuracy.[20] As all RMSD values in this study met this criterion, the docking protocol was validated for subsequent simulations. Molecular docking simulations were performed to evaluate the interaction profiles of selected flavonoid compounds with key protein targets identified through the previous PPI network analysis. Based on this analysis, ER- α (PDB ID: 3ERT), BCL-2 (PDB ID: 6GL8), and PARP1 (PDB ID: 4R6E) were selected due to their established roles in breast cancer signaling and apoptosis regulation. Six flavonoid ligands, along with each target's native ligands were docked to assess their comparative binding affinities and interaction profiles. Across all targets, the evaluated flavonoids formed consistent hydrogen bonds with key residues such as Glu- 353 and His-524 (ER-α), Ala-149 and Glu-136 (BCL-2), and Asp-766 and Gly-863 (PARP1) supported by extensive hydrophobic

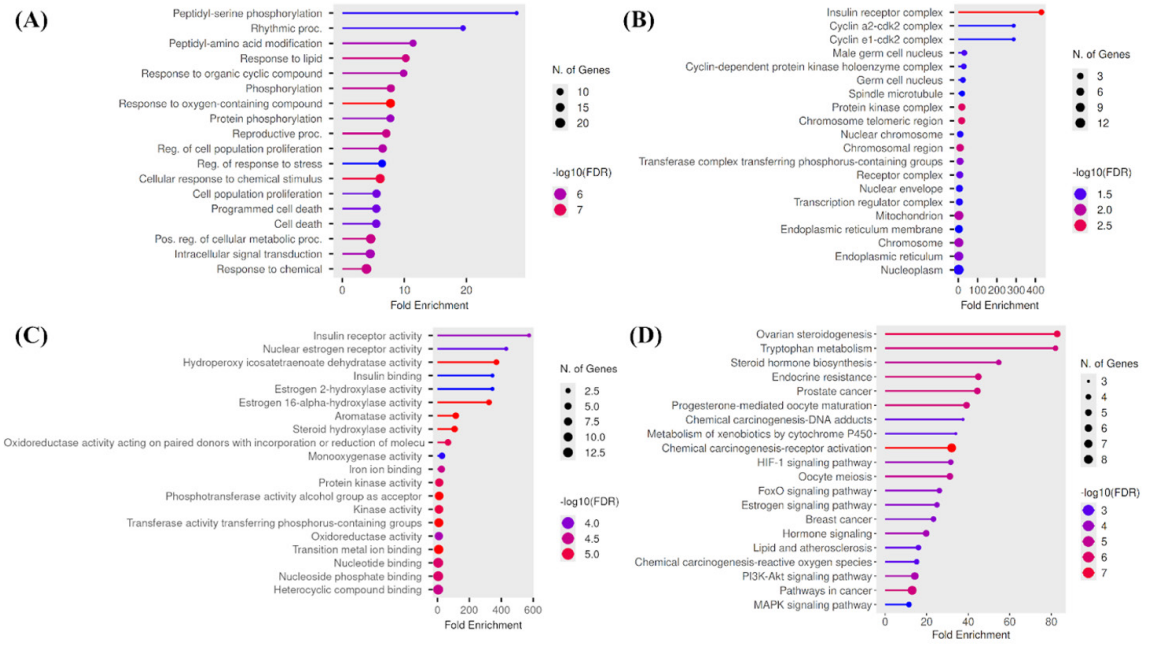

Figure 4. GO analysis of 26 breast cancer targets (**A**) Biological process, (**B**) Cellular components, (**C**) Molecular function, and (**D**) KEGG pathway analysis

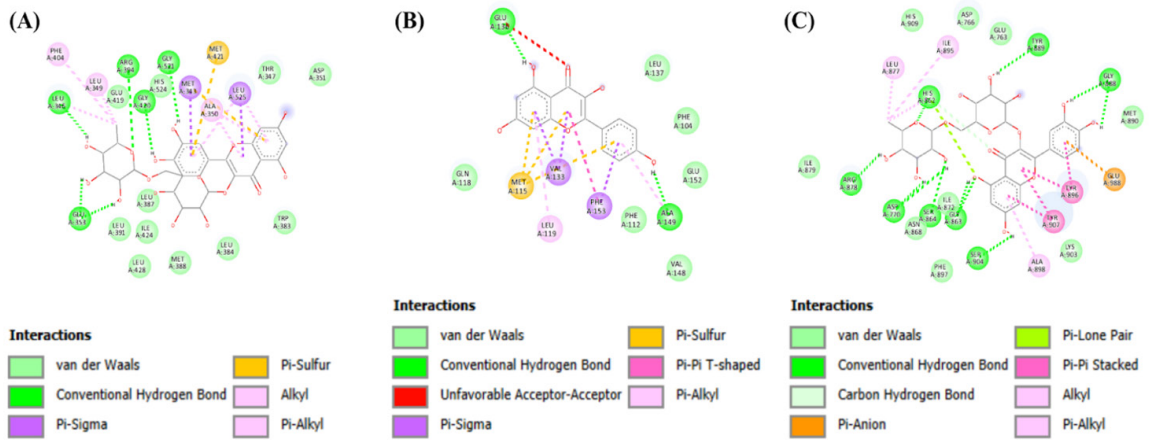

Figure 5. Visualization of molecular docking result (**A**) Rutin against ER-α; (**B**) Kaempferol against BCL-2; (**C**) Rutin against PARP1

and aromatic interactions. Pi-Alkyl, pi-sigma, and occasional pi-sulfur contacts further contributed to complex stability as seen in Table 3. Overall, the docking results showed rutin as the most promising flavonoid with strong binding energy and lower inhibition constant towards ER-α (-8.28 kcal/mol and 853.84 nM) and PARP1 (- 11.14 kcal/mol and 6.85 nM), followed by kaempferol towards BCL-2 which exhibited binding energy value of -5.94 kcal/mol and inhibition constant of 43.91 μM, visualized in Figure 5. The ligand-receptor binding affinities are summarized in Table 3.

4. Discussion

In this study, an integrated in silico analysis was conducted to explore the anti-breast cancer potential of selected flavonoids through drug-likeness screening using Lipinski's Rule of Five (RO5), ADME-Tox profiling, network pharmacology, and molecular docking simulations. Evaluation of the evaluated flavonoids using Lipinski's Rule of Five (RO5) indicates that quercetin, kaempferol, catechin, epicatechin, and luteolin possess favorable drug- likeness, satisfying all RO5 criteria related to molecular weight, lipophilicity, and hydrogen-bonding capacity, which support intestinal absorption and membrane permeability.[21,22] In contrast, rutin violates multiple RO5 parameters, including excessive molecular weight and hydrogen-bonding features, along with a negative Log P value indicating high hydrophilicity, leading to poor membrane permeability, low intestinal absorption, and limited oral bioavailability.[23] In fact, rutin is a glycoside that will be hydrolyzed into quercetin by intestinal enzymes. This metabolic conversion process is the reason why rutin is absorbed slowly in the intestines.[24] Despite its pharmacological potential, rutin's clinical application is constrained by poor solubility and pharmacokinetics, necessitating formulation-based strategies to enhance its delivery.[25] Nanocarrier systems, such as folic acid-conjugated keratin nanoparticles and rutin-loaded zinc oxide nanoparticles, have demonstrated improved cellular uptake and enhanced cytotoxic activity in MCF-7 breast cancer cells, supporting their ability to improve rutin's therapeutic efficacy.[26,27] ADMET analysis further revealed distinct pharmacokinetic and safety profiles among the flavonoids. Kaempferol and luteolin showed favorable oral bioavailability with high intestinal absorption and acceptable permeability but were predicted to be mutagenic, with kaempferol also exhibiting carcinogenic potential in rat models. Conversely, rutin displayed poor pharmacokinetic performance, characterized by very low intestinal absorption and limited blood-brain barrier penetration, yet consistently showed a favorable safety profile as a non-mutagenic and non-carcinogenic compound, highlighting its potential as a safe therapeutic candidate if its pharmacokinetic limitations can be effectively addressed.

Table 3. Molecular Docking Result from the Evaluated Flavonoid Compounds

Compounds	ER-α (3ERT)				BCL-2 (6GL8)				PARP1 (4R6E)			
	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	Hydrogen Bonds	Other Bonds	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	Hydrogen Bonds	Other Bonds	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	Hydrogen Bonds	Other Bonds
4-HYDROXYTAMOXIVIEN (native ligand)	-11.9	1.88 nM	Glu-353	Met-421 Leu-525 Ala-350 Leu-387 Leu-391 Phe-404 Leu-346 Thr-347	-	-	-	-	-	-	-	-
S55746 (native ligand)	-	-	-	-	-12.59	589.85 µM	Ala-149	Asp-111 Arg-146 Phe-104 Leu-137 Val-133 Leu-119 Met-115 Phe-112 Tyr-108	-	-	-	-
Niraparib (native ligand)	-	-	-	-	-	-	-	-	-10.26	30.02 nM	Asp-766 Gly-863	Ala-898 Lys-903 Tyr-896 Tyr-907 Tyr-889
Quercetin	-7.58	2.78 µM	Glu-419 Met-343 His-524 Glu-353	Met-421 Met-388 Leu-346 Leu-391 Ala-350 Leu-387	-5.29	131.53 µM	Ala-149 Gln-118 Glu-136	Phe-153 Val-133 Met-115	-9.05	231.06 nM	Gly-888 Glu-988 Lys-903 Trp-861	Tyr-889 His-862 Tyr-907 Tyr-896 Ala-89

Kaempferol	-7.61	2.62 µM	Gly-521 His-524 Leu-387 Glu-353	Met-388 Leu-346 Ala-350	-5.94	43.19 µM	Glu-136 Ala-149	Met-115 Leu-119 Val-133 Phe-153	-8.45	637.98 nM	Met-890 Glu-988 Trp-861 Ser-904 Gly-863 Gly-888	His-862 Tyr-907 Tyr-896 Tyr-889
Catechin	-7.45	3.48 µM	Glu-419 Met-343 Thr-347 Gly-521	Ile-424 Leu-525 Met-421 Ala-350	-5.8	56.22 µM	Tyr-108 Ala-149 Glu-136	Met-115 Leu-137 Phe-153 Val-133 Phe-112 Phe-104	-7.34	4.17 µM	Gly-894 Tyr-896 Gly-863 Ser-904 Ile-895	Tyr-896 Tyr-907 His-862
Epicatechin	-7.75	2.1 µM	Leu-387 Glu-419 His-524 Glu-353	Leu-346 Leu-525 Met-421 Met-388 Leu-391	-5.07	191.11 µM	Glu-136 Ala-149	Met-115 Leu-137 Val-133	-8.59	504.39 nM	Ser-904 Glu-988 Gly-888 Gly-863	Ala-898 Glu-988 Tyr-896 Tyr-907 Lys-903 His-862
Luteolin	-8.0	1.37 µM	Arg-394 Glu-353 Met-343 Thr-347	Leu-391 Leu-387 Leu-384 Leu-525 Ala-350	-5.56	84.21 µM	Ala-149 Gln-118 Glu-136	Phe-153 Met-115 Val-133	-9.08	220.79 nM	Lys-903 Glu-988 Gly-888 Met-890 Gly-863	Tyr-907 Glu-988 Tyr-896 His-862
Rutin	-8.28	853.84 nM	Glu-353 Leu-346 Arg-394 Gly-420 Gly-521	Phe-404 Leu-349 Ala-350 Met-343 Leu-525	-5.86	50.92 µM	Ala-149 Glu-136 Asp-111	Met-115 Leu-137 Tyr-108 Phe-104	-11.14	6.85 nM	His-862 Tyr-889 Gly-888 Ser-904 Gly-863 Ser-864 Asp-770 Arg-878	Leu-877 Ile-895 His-862 Glu-988 Tyr-896 Tyr-907 Ala-898

Following the screening process, a total of 27 overlapping targets were identified for the flavonoid compounds and breast cancer, which demonstrates the broad therapeutic potential of these bioactive candidates. From this interaction network, three core targets were further determined using topological analysis, among which ER-α, BCL-2, and PARP1 emerged as the most significant targets due to their high degree of connectivity. These core targets were deeply involved in the regulation of breast cancer progression. ER-α is a nuclear receptor that functions as a critical regulator of cellular proliferation, differentiation, and survival in hormone-dependent (ER-positive) breast cancer, which accounts for approximately 70% of all cases, often facilitating tumor growth through genomic signaling pathways wherein the activated receptor binds to estrogen-responsive elements (EREs) to drive the transcription of oncogenic target genes such as Cyclin D1, MYC, and BCL-2.[28–30] Concurrently, BCL-2 family proteins, which localize not only to mitochondria but also to the endoplasmic reticulum (ER) and nuclear membrane, functions as a potent anti-apoptotic regulators that secure cancer cell survival by inhibiting the mitochondrial apoptotic pathway, specifically by restraining pro-apoptotic effectors like BAX and BAK. In breast cancer, high expression of BCL-2 is associated with prolonged cell cycles which paradoxically delays tumor cell proliferation, but ultimately foster resistance against cytotoxic treatments such as chemotherapy and promotes metastatic progression by preventing apoptosis even when cells are damaged.[31,32] Simultaneously, PARP1 plays a critical role in maintaining genomic stability, where its regulatory function in DNA repair, especially in the base excision repair pathway, enables cancerous cells to recover and survive DNA-targeting therapies. This recovery is mediated by PARylation, a post translational modification involving the transfer of ADP-ribose units from NAD+ to form linear or branched polymer chains on target proteins. Through this process, PARP1 creates a scaffold that reorganized local nucleoprotein structures to recruit repair factors and undergoes auto-modification, which is indispensable for its dissociation from DNA damage sites to allow the repair to finish. Consequently, PARP1's ability to facilitate DNA damage repair through these dynamic mechanisms, combined with its interaction with chromatin remodeling factors, contributes to the resistance mechanisms of tumors under therapeutic stress.[33–36]

These targets were subsequently validated using molecular docking analysis. The docking results revealed favorable intermolecular interactions between the flavonoids and their respective targets, with all binding energies exhibited below -5.0 kcal/mol.[37] Therefore, the molecular docking results were regarded as having a spontaneous and stable binding between the flavonoids and the targets.[38] According to the molecular docking results, rutin demonstrated promising binding toward ER-α and PARP1 with binding energy value of -8.28 kcal/mol and -11.14 kcal/mol respectively along with inhibition constant value of 853.84 nM and 6.85 nM, which are the lowest among the evaluated flavonoids. Furthermore, rutin showed conventional hydrogen bond across amino acid residue Glu-353 in ER-α and Gly-863 in PARP1 which are the key interaction for potential inhibitory activity on each targets.[39,40] Similar interactions were also observed in 4-hydroxytamoxifen and niraparib which serves as their respective native ligand and standardized reference drug for each targets, thereby suggesting that rutin may exert corresponding mechanism in inhibiting ER-α and PARP1 and potentially modulate estrogen receptor signaling and PARP1-mediated DNA repair pathways in breast cancer.[41,42] On the other hand, kaempferol revealed similar interactions with S55746 as BCL-2 potent inhibitor and also its respective native ligand through conventional hydrogen bond at Glu-136 residue in BCL-2, while also having the lowest binding energy (-5.94 kcal/mol) and inhibition constant (43.91 μM) compared to the other evaluated flavonoids.[43] This finding indicates that kaempferol may interact with BCL-2 comparable to the established inhibitor which potentially wields similar modulation effects toward BCL-2-mediated anti-apoptotic

signaling in breast cancer.[44]

GO enrichment analysis revealed the direct targeting of cell cycle machinery, which is frequently dysregulated in aggressive breast cancer subtypes. The enrichment of cyclin A2-CDK2 and cyclin E1-CDK2 complexes in GO-CC suggest that the flavonoid compound may exert its anti-proliferative effects by acting as a cell cycle inhibitor, potentially inducing arrest at the G1/S checkpoint, which is critical for preventing uncontrolled division of cancer cells.[45,46] A previous study by Turner et al. (2019) demonstrated that high CCNE1 expression limits the efficacy of CDK4/6 inhibitor in hormone receptor positive metastatic breast cancer, thereby underscoring the clinical necessity of agents that can directly inhibit the cyclin E/CDK2 axis to overcome such resistance mechanisms.[47] Furthermore, the modulation of peptidyl-serine phosphorylation that showed high enrichment in GO-BP indicates the flavonoid's ability to modulate kinase activity that may interfere with estrogen receptor alpha. Phosphorylation at specific serine residues is a critical sensor that integrates growth factor signaling, such as PI3-Akt and MAPK, to regulate ER-α transcriptional activity and tumor progression. This finding aligns with the KEGG pathway analysis, which highlights the enrichment of the PI3-Akt and MAPK signaling pathways.[48,49] Crucially for breast cancer therapy, the GO-MF results exhibit significant interaction with nuclear estrogen receptor activity that aligns with the estrogen signaling pathway in KEGG pathway analysis. This suggests potential efficacy as a selective modulator for ER-positive breast cancer subtypes. The regulation of ER-α requires cooperation with other nuclear receptors to bind to super enhancers and drive gene expression. Consequently, targeting the estrogen receptor could disrupt the critical transcriptional machinery.[50] Our KEGG analysis also identified endocrine resistance as a key target pathway. Previous study reported that disrupting the interaction within the estrogen signaling axis can effectively re- sensitize Tamoxifen-resistant cells to endocrine therapy, which suggests that flavonoid bioactive compound holds potential as an agent to overcome drug resistance.[50,51]

Although the in silico analyses offer initial insights into the potential therapeutic properties of flavonoids, the findings are still predictive in nature and should be interpreted with caution. Nevertheless, the reliability of our computational model for rutin is supported by prior in silico and in vitro studies. For instance, Permana et al. (2024) demonstrates strong binding affinities for ER-α (-338.8 kcal/mol), a finding validated by a significant reduction in ER-α expression in T47D breast cancer cells.[52] Similarly, Pravin et al. (2024) corroborated high-affinity in silico scores for rutin against PARP1 (-12.4 kcal/mol) with in vitro evidence of selective cytotoxicity and targeted DNA damage in cancer cell models.[53] Furthermore, a review by Pandey et al. (2021) highlights that the biological plausibility of rutin's activity is reinforced by its role in regulating the BCL-2 family. Experimental studies have shown that rutin induces mitochondrial-mediated apoptosis by suppressing anti-apoptotic BCL-2 and decreasing the BCL-2/BAX ratio across various carcinoma models.[54] Despite these supportive indications, the absence of molecular dynamics simulations and experimental verification, specifically in vitro or in vivo, constrains the confirmation of the stability and biological significance of the predicted ligand-receptor interactions. Consequently, further studies integrating these validation approaches are necessary to strengthen the proposed mechanisms and therapeutic interpretations.

5. Conclusion

The in silico evaluation of six flavonoid compounds suggests their potential as therapeutic agents targeting key molecular pathways in breast cancer, specifically ER-α, BCL-2, and PARP1. Among the evaluated flavonoid compounds, rutin emerged the most promising candidate, demonstrating the highest binding affinity to both PARP1 (binding energy = -11.14 kcal/mol, Ki = 6.85 nM) and ER-α (binding energy = -8.28 kcal/mol, Ki = 853.84 nM). These interactions suggest rutin may potentially modulate estrogen signaling and disrupts DNA repair mechanisms essential

for cancer cell survival. Although rutin does not fully meet Lipinski's Rule of Five criteria due to bioavailability limitations, its favorable safety profile supports its potential for further development, particularly through formulation in advanced drug delivery systems. On the other hand, luteolin and kaempferol displayed high binding activity and favorable oral bioavailability. However, while kaempferol showed notable inhibition of BCL-2, its predicted carcinogenicity in rodent models warrants cautious evaluation. Overall, these findings indicate that rutin suggest its potential as preliminary therapeutic leads. Further, additional studies, including molecular dynamics, in vitro, and in vivo experiments as well as pharmacodynamic and toxicity evaluations, are required to confirm their efficacy and safety for potential use in breast cancer treatment.

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