RUBIADIN AS A MULTI-TARGET NATURAL AGENT AGAINST BREAST CANCER: INSIGHTS FROM NETWORK PHARMACOLOGY AND MOLECULAR DOCKING

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ABSTRACT

Breast cancer is a complex and heterogeneous malignancy characterized by dysregulated signaling pathways, uncontrolled cell proliferation, and resistance to apoptosis, posing significant challenges to effective therapy. Natural compounds with multi-target potential have emerged as promising alternatives for cancer management. Rubiadin, a bioactive anthraquinone derived from Rubia species, has demonstrated various pharmacological activities; however, its molecular mechanisms against breast cancer remain largely unexplored. In the present study, an integrated network pharmacology and molecular docking approach was employed to elucidate the potential anticancer mechanisms of Rubiadin against breast cancer. Potential Rubiadin-related targets were identified using SwissTargetPrediction and Similarity Ensemble Approach tools, while breast cancer–associated genes were retrieved from the GeneCards database. Overlapping targets were analyzed to construct a protein–protein interaction (PPI) network, followed by hub protein identification using Cytoscape. Functional enrichment analysis was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to uncover key biological processes and signaling pathways. The results revealed 147 common targets between Rubiadin and breast cancer, with a STRING-filtered PPI network comprising 93 nodes and 476 edges. GO and KEGG analyses highlighted the significant involvement of apoptosis, protein phosphorylation, and the PI3K-Akt signaling pathway. Molecular docking demonstrated strong binding affinity of Rubiadin toward key hub proteins, particularly ESR1, EGFR, MTOR, BCL2, and HSP90AA1. Overall, this study provides mechanistic insights into the multi-target anticancer potential of Rubiadin and supports its therapeutic relevance against breast cancer.

Keyword: Cancer, Rubiadin, PI3K-Akt Pathway, Network Pharmacology, Molecular Docking.

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INTRODUCTION

Breast cancer remains one of the most prevalent and life-threatening malignancies among women worldwide, accounting for a substantial proportion of cancer-related morbidity and mortality. According to global cancer statistics, breast cancer has surpassed all other cancers in incidence, with a continuously rising burden in both developed and developing countries. Despite significant advances in early diagnosis and therapeutic strategies, including surgery, chemotherapy, radiotherapy, hormone therapy, and targeted molecular treatments, the overall clinical outcomes remain unsatisfactory for many patients [1]. The emergence of drug resistance, severe adverse effects, tumor heterogeneity, and disease recurrence poses major challenges in effective breast cancer management [2]. Consequently, there is an urgent need to explore novel therapeutic agents with multi-target potential, improved safety profiles, and enhanced efficacy.

Breast cancer is a complex and multifactorial disease characterized by dysregulated cell proliferation, resistance to apoptosis, altered metabolism, chronic inflammation, oxidative stress, and aberrant intracellular signaling pathways. Among these, the phosphatidylinositol-3-kinase/protein kinase B (PI3K-Akt) signaling pathway plays a pivotal role in breast cancer initiation, progression, metastasis, and therapy resistance. Hyperactivation of this pathway promotes tumor cell survival, proliferation, angiogenesis, and metabolic reprogramming, while inhibiting programmed cell death [3], [4], [5]. Key proteins such as EGFR, MTOR, BCL2, ESR1, GSK3B, and HSP90AA1 are critically involved in PI3K-Akt signaling and are frequently dysregulated in breast cancer. Therefore, targeting multiple components of this pathway has emerged as a promising therapeutic strategy.

Natural products and phytochemicals have gained considerable attention as potential anticancer agents due to their structural diversity, biological compatibility, and ability to modulate multiple molecular targets simultaneously [6]. Several plant-derived compounds have demonstrated significant anticancer activity by regulating cell cycle arrest, apoptosis, inflammation, oxidative stress, and signal transduction pathways [7]. Unlike conventional single-target drugs, phytochemicals often exert pleiotropic effects, making them particularly suitable for complex diseases such as cancer.

Rubiadin (1,3-dihydroxy-2-methylanthraquinone) is a naturally occurring anthraquinone compound primarily isolated from plants of the *Rubia* species,

including *Rubia cordifolia*, which has been extensively used in traditional medicinal systems [8]. Previous studies have reported diverse pharmacological activities of Rubiadin, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Experimental evidence suggests that Rubiadin can inhibit cancer cell proliferation, induce apoptosis, and modulate oxidative stress and inflammatory responses [9]. However, the precise molecular mechanisms underlying its anticancer potential, particularly against breast cancer, remain poorly understood. A comprehensive investigation into its target proteins, signaling pathways, and molecular interactions is essential to validate its therapeutic relevance.

In recent years, network pharmacology has emerged as a powerful and systematic approach to elucidate the complex interactions between drugs, targets, pathways, and diseases. Unlike conventional pharmacological methods that focus on a single target, network pharmacology integrates bioinformatics, systems biology, and computational tools to uncover the multi-target and multi-pathway mechanisms of bioactive compounds. This approach is especially suitable for studying phytochemicals, which often exert their effects through coordinated modulation of multiple molecular networks [10]. By constructing drug–target–disease interaction networks, network pharmacology enables the identification of key hub proteins and critical signaling pathways involved in disease modulation.

Complementing network pharmacology, molecular docking is a widely used computational technique to predict the binding affinity and interaction patterns between small molecules and target proteins at the atomic level. Docking analysis provides valuable insights into ligand–protein interactions, binding energies, and key amino acid residues involved in complex stabilization [11]. When combined with network pharmacology, molecular docking strengthens mechanistic predictions and enhances the reliability of in silico findings.

In the context of breast cancer, the integration of network pharmacology and molecular docking offers a robust framework to explore how natural compounds such as Rubiadin interact with multiple oncogenic targets and signaling pathways. Identifying hub proteins within protein–protein interaction (PPI) networks can highlight critical molecular nodes that govern disease progression. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis, further aid in understanding the biological processes, cellular components, and molecular functions associated with these targets.

Given the pivotal role of the PI3K-Akt signaling pathway in breast cancer pathophysiology, investigating whether Rubiadin can modulate this pathway through interaction with key regulatory proteins is of particular interest [12]. Proteins such as EGFR, MTOR, ESR1, BCL2, and HSP90AA1 not only regulate tumor growth and survival but also contribute to therapeutic resistance and poor prognosis. Targeting these proteins simultaneously may offer a more effective strategy to overcome resistance and improve treatment outcomes.

Therefore, the present study aims to systematically investigate the potential molecular mechanisms of Rubiadin against breast cancer using an integrated network pharmacology and molecular docking approach. Initially, potential Rubiadin-related targets and breast cancer-associated genes were identified using publicly available databases. Overlapping targets were determined to construct a protein–protein interaction network, followed by hub protein identification. Functional enrichment analyses were performed to elucidate the key biological processes and signaling pathways involved. Furthermore, molecular docking was employed to validate the binding affinity of Rubiadin with key hub proteins associated with the PI3K-Akt signaling pathway.

2. MATERIALS & METHODS

2.1 Identification of drug related targets and disease related targets

The canonical SMILES representation of Rubiadin was retrieved from the PubChem database [13], and it was used as an input for target prediction using the SwissTargetPrediction tool and Similarity Ensemble Approach (SEA) tool [14], [15]. These tools predict the potential protein targets for a given compound based on 2D and 3D similarity measures. With the help of GeneCards database [16], proteins linked with Breast Cancer were identified. A comprehensive search for "Breast cancer" was performed, and the resulting gene list was compiled.

2.2 Overlapping target identification

To pinpoint the specific pathways through which Rubiadin exert its effects, the predicted drug targets and the Breast Cancer-related targets were compared. A Venn diagram was generated using the Bioinformatics.psb [17] tool to identify common targets present in both the lists.

2.3 Protein-protein interaction network construction and visualization

Step 1: The common targets identified with Bioinformatics.psb tool were then used to construct a protein-protein interaction map using the STRING database [18], [19]. This PPI network was then exported to Cytoscape for visualization and further analysis.

Step 2: Within Cytoscape, topological analysis was performed to identify the hub proteins using the Cytohubba built-in programme [20]. The hub targets were built in a separate subnetwork, and the proteins with a high degree of connectivity within the network suggests a central role in the disease mechanism.

2.4 Functional enrichment analysis of common targets

To gain further insight into the biological roles of the common targets, functional enrichment analysis was performed using DAVID software [21]. The common gene symbols were uploaded, and the annotation tool was run to obtain GO terms and KEGG pathways.

2.5 Molecular docking examination

The PubChem database was used to download the 3D structure of Rubiadin, which was then converted to PDB format using Discovery Studio Visualizer. Ligand preparation was performed using AutoDock Tools (v1.5.7) [22], where Gasteiger charges were assigned, rotatable bonds were defined, and the structure was saved in PDBQT format [22], [23]. The target protein structures were downloaded from the RCSB PDB database [24]and prepared by removing waters and heteroatoms, then adding polar hydrogens plus Kollman charges.

Molecular docking was performed in AutoDock 4.2 using Lamarckian Genetic Algorithm [25]. Results of docking were analyzed with respect to binding energy (kcal/mol), and the most stable ligand-protein complexes were used for further analysis through visualization and interactions assessment in Discovery Studio Visualizer.

3. RESULTS

3.1Identification of overlapping targets

Using SwissTarget prediction tool and Similarity Ensemble Approach (SEA), a total of 189 proteins were identified as potential targets of Rubiadin. GeneCards analysis revealed 160,601 genes / proteins associated with Breast cancer. Comparison of these datasets identified 147 overlapping proteins, as visualized in the Venn diagram (Figure1).

3.2Protein-Protein Interaction Network Analysis

A PPI network was constructed in STRING using the 147 common target proteins. After applying STRING confidence scores filtering, the refined PPI network consisted of 93 nodes and 476 edges as depicted in Figure 2, indicating a significant degree of interaction among these proteins. Subsequently, the resulting PPI data was exported to Cytoscape for further analysis. Within Cytoscape, node scores were calculated, and the top 10 protein targets were selected based on their degree scores and the results are shown in Table 1. The interaction among topmost 15 selected protein targets are shown in Figure 3.

3.3 Gene Ontology Analysis

Gene ontology enrichment analysis using DAVID software revealed significant enrichment for several biological processes. A total of 372 Biological Processes (BP), 56 Cellular Components (CC) and 104 Molecular Functions (MF) were enriched. The top 10 terms within each category were identified by applying filters (p-value ≤ 0.01 and gene count ≥ 5). Table 2 summarizes the prioritized biological processes, while the corresponding cellular components and molecular functions are described in Table 3 and Table 4, respectively. Additionally, a bar plot visualization of GO results is shown in Figure 4, displaying the enriched biological processes, cellular components, and molecular functions that are associated with Rubiadin's breast cancer–associated targets.

3.4 KEGG Pathway Investigation

The KEGG pathway analysis revealed that the common protein targets are involved in several crucial biological pathways, suggesting their diverse roles in cellular functions and disease pathogenesis. Out of 138 pathways analyzed, 10 signaling pathways were selected (Table. 5) by applying filters (p-value ≤ 0.01 and gene count ≥ 5). Among them the most enriched pathways are Central carbon metabolism in cancer, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt Signaling Pathway, Pathways in Cancer, Endocrine resistance, and HIF-1 signaling pathway, suggesting Rubiadin's potential to modulate the key intracellular signaling cascades that are involved in Cell survival, proliferation, apoptosis resistance. Figure 5 illustrates the PI3K-Akt signaling pathway, which plays an important role in mediating cellular responses to oxidative stress, inflammation, and apoptosis, these processes are linked to Breast Cancer. The ability of Rubiadin to modulate the key proteins such as BCL2, EGFR, ESR1, GSK3B, reveals its potential to regulate the inflammatory responses and oxidative stress, offering a protective effect against breast cancer. The linkage between the topmost enriched pathways and their corresponding genes are depicted in Figure 6 by Sankey and dot plot.

Molecular Docking of Rubiadin with PI3K-Akt signaling pathway -Associated Targets

Rubiadin was docked with the key hub proteins like MTOR, EGFR, BCL2, ESR1, and HSP90AA1, which play critical roles in breast cancer progression and are directly involved in the PI3K-Akt signaling pathway. These proteins were selected for docking from the top 10 hub proteins based on their direct involvement in the PI3K-Akt signaling pathway and their topological importance within the PPI network. The binding energy results obtained are shown in Table 6, and suggests that Rubiadin binds moderately across all selected PI3K-Akt -associated proteins. Among them, Rubiadin exhibited the highest binding affinity with ESR1 (–8.9 kcal/mol), indicating a strong interaction. Additionally, Rubiadin showed strong binding affinity toward EGFR (–8.8 kcal/mol) and HSP90AA1 (–7.7 kcal/mol), BCL2 (–7.6 kcal/mol) and MTOR (–7.9 kcal/mol). Figures 7 (a-f) illustrates the 2D interaction diagrams that shows specific residues involved in the ligand binding and the types of interactions stabilizing the complexes.

Table 1: Topmost 15 protein targets ranked by degree method identified from the STRING filtered PPI network

S. No	Gene Symbol	Name	Degree Score
1	EGFR	Epidermal Growth Factor Receptor	64
2	BCL2	BCL2 Apoptosis Regulator	63
3	ESR1	Estrogen Receptor 1	58
4	SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase	57
5	HSP90AA1	Heat Shock Protein 90 Alpha Family	57

		Class A Member 1	
6	HSP90AB1	Heat Shock Protein 90 Alpha Family Class B Member 1	53
7	MTOR	Mechanistic Target of Rapamycin Kinase	50
8	MMP9	Matrix Metallopeptidase 9	45
9	GSK3B	Glycogen Synthase Kinase 3 Beta	42
10	PTGS2	Prostaglandin-Endoperoxide Synthase 2	40

Table 2: Top 10 enriched biological processes (BP), identified through Gene Ontology analysis (p ≤ 0.01 and gene count ≥5).

S. No	Term	Gene Count	PValue
1	Protein phosphorylation	50	4.20E-23
2	Phosphorylation	52	1.70E-23
3	Reg. of programmed cell death	58	3.60E-25
4	Reg. of apoptotic proc.	56	2.00E-24
5	Response to oxygen-containing compound	58	2.00E-24
6	Cellular response to chemical stimulus	67	7.60E-25
7	Programmed cell death	61	2.50E-22
8	Phosphate-containing compound metabolic proc.	69	2.00E-24
9	Phosphorus metabolic proc.	69	2.00E-24
10	Response to chemical	93	1.70E-28

Table 3: Top 10 enriched cellular components (CC), identified through Gene Ontology analysis (p ≤ 0.01 and gene count ≥5).

S. No	Term	Count	PValue
1	Phosphatidylinositol 3-kinase complex class ia	4	7.40E-06
2	Ficolin-1-rich granule lumen	10	1.70E-06
3	Membrane raft	14	1.50E-06
4	Membrane microdomain	14	1.50E-06
5	Vesicle lumen	16	5.80E-07
6	Secretory granule lumen	15	1.50E-06
7	Cytoplasmic vesicle lumen	15	1.50E-06
8	Synapse	31	7.40E-06
9	Cell junction	44	2.20E-07
10	Extracellular space	50	1.50E-06

Table 4: Top 10 enriched molecular functions (MF), identified through Gene Ontology analysis (p ≤ 0.01 and gene count ≥5).

S. No	Term	Gene Count	PValue
1	Protein kinase activity	33	1.20E-18
2	Phosphotransferase activity alcohol group as acceptor	36	3.00E-19
3	Kinase activity	36	5.80E-18
4	Transferase activity transferring phosphorus-containing groups	37	3.80E-16
5	Catalytic activity acting on a protein	75	6.80E-30
6	Adenyl nucleotide binding	47	1.40E-15
7	Heterocyclic compound binding	56	6.90E-16
8	Nucleotide binding	52	1.70E-14
9	Nucleoside phosphate binding	52	2.20E-14
10	Hydrolase activity	58	1.40E-15

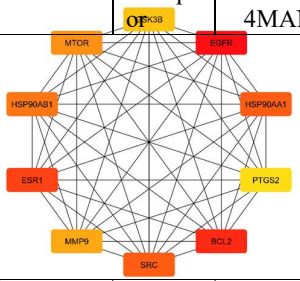
Table 5: Top 10 KEGG Pathways, that are common to Rubiadin and Breast Cancer (p ≤ 0.01 and gene count ≥5).

S. No	Term	Gene Count	PValue
1	Central carbon metabolism in cancer	14	1.90E-15
2	EGFR tyrosine kinase inhibitor resistance	14	6.40E-15
3	Endocrine resistance	13	1.60E-12
4	Prostate cancer	13	1.60E-12
5	HIF-1 signaling pathway	13	4.70E-12
6	Relaxin signaling pathway	14	2.90E-12
7	Ras signaling pathway	17	4.70E-12
8	Pathways in cancer	38	5.80E-27
9	MicroRNAs in cancer	21	3.60E-14

10	PI3K-Akt signaling pathway	23	6.40E-15

Table 6: Binding affinities of Rubiadin with six PI3K-Akt signaling pathway-related target proteins identified for docking analysis.

S. No	Gene ID	Target Name	PDB ID	Binding affinity (kcal/mol)
1	MTOR	Mechanistic Target of Rapamycin Kinase	4JSV	-7.9
2	EGFR	Epidermal Growth Factor Receptor	4MAN	-7.6
3	ESR1	Estrogen Receptor 1	3T0Z	-7.7
4	HSP90AA1	Heat Shock Protein 90 Alpha	3ERT	-8.8
5	BCL2	BCL2 Apoptosis Regulator	1M17	-8.9



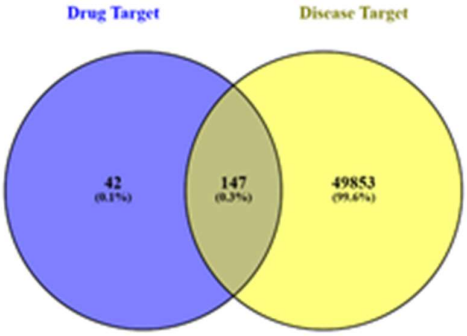


Figure 1. Venn diagram showing overlap between Rubiadin targets and genes related to Breast Cancer.

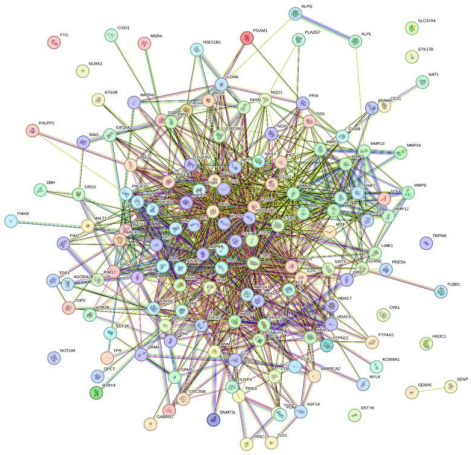
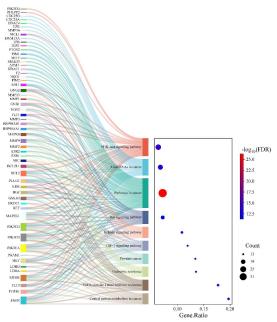


Figure 2. Protein-protein interaction network of 93 common protein targets between Rubiadin and Breast Cancer constructed using STRING.

Figure 3. Top 15 hub proteins ranked by degree. The degree score is presented using node color, with values displayed in descending order from red (highest score) to yellow (lowest score).



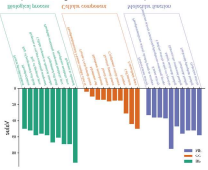


Figure 4. Top 10 GO Terms Biological processes, cellular components and molecular functions along with their gene counts.

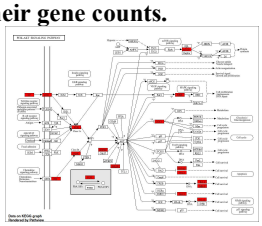


Figure 5. Localization of core protein targets across PI3K-Akt signaling pathway. Nodes in red represents the shared proteins between Rubiadin and Breast Cancer.

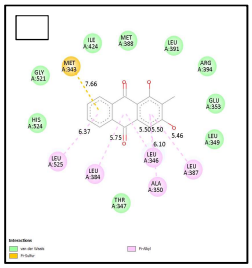


Figure 6. Sankey and Dot Plot indicating the topmost enriched KEGG pathways along with their associated proteins.

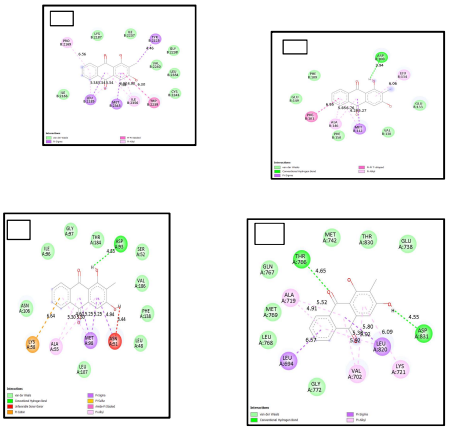


Figure 7. 2D interaction diagrams showing the binding results of Rubiadin with five PI3K-Akt signaling pathway-related target proteins: (a) MTOR, (b) BCL2, (c) HSP90AA1, (d)ESR1, (e) EGFR

The molecular docking analysis indicated that Rubiadin was found to show positive binding affinities in all the chosen hub proteins. Rubiadin was found to have a binding affinity of -7.9 kcal / molar in mTOR and was stabilized predominantly through hydrophobic interactions, including π-π T-shaped interaction with Tyr2225 (4.46 Å) and multiple π-π stacked and π-alkyl interactions with residues including Leu2185, Met2345, Ile2356, and Trp2239, which suggested that it BCL-2 , the ligand showed stronger binding affinity of -8.9 kcal/mol, with a conventional hydrogen bond with Asp108 (3.94 Å) along with π-π T-shaped interaction with Phe101 and π-sigma interactions with Met112 supported by additional π-alkyl contacts with Ala146 and Leu134, suggesting effective stabilization within the anti-apoptotic binding groove.

In the case of HSP90AA1, Rubiadin exhibited binding affinity of -8.8 kcal/mol and a conventional hydrogen bond with Asp93 (4.83 Å) and π-sulfur bond with Met98, amide- - stacked bond with Ala55 and Met98, and -alkyl bond with Leu107, which demonstrates that Rubiadin is highly stabilized in the ATP-binding region of the chaperone protein. The binding affinity of the ligand in ESR1 was -7.7 kcal/mol, with binding largely dominated by hydrophobic force, with a 7.66 Å interaction between the ligand and Met343 and several π-alkyl interactions with residues such as Leu525, Leu384, Leu346, Leu387, Leu349, and Ala350. Finally, Rubiadin had a binding affinity of -7.6 kcal/mol with two standard hydrogen bonds formed (4.65 Å and 4.55 Å) with Thr766 and Asp831 along with π-sigma interactions with Leu694 and Ala719 and π-alkyl interactions involving Val702 and Leu820, which collectively stabilize the ligand within the kinase active site.

Discussion

The present study employed an integrated network pharmacology and molecular docking approach to elucidate the potential molecular mechanisms underlying the anticancer effects of Rubiadin against breast cancer. Given the multifactorial nature of breast cancer, characterized by dysregulated signaling networks and therapeutic resistance, the identification of multi-target agents is of considerable clinical relevance.

Network pharmacology analysis revealed 147 overlapping targets between Rubiadin and breast cancer, suggesting its broad regulatory potential. The STRING-filtered protein–protein interaction network highlighted key hub proteins, including EGFR, MTOR, ESR1, BCL2, and HSP90AA1, which are critically involved in tumor growth, survival, and resistance mechanisms. Functional enrichment analysis further demonstrated significant involvement of biological processes related to apoptosis, protein phosphorylation, and cellular responses to chemical stimuli. KEGG pathway analysis identified the PI3K-Akt signaling pathway as a central regulatory axis, underscoring its pivotal role in breast cancer progression and therapy resistance.

Molecular docking analysis supported the network-based predictions by demonstrating favorable binding affinities of Rubiadin with major PI3K-Akt pathway–associated proteins. Notably, strong interactions with ESR1 and EGFR suggest Rubiadin's potential to modulate hormone-dependent and growth factor–mediated signaling in breast cancer. The ability of Rubiadin to interact with multiple oncogenic targets highlights its promise as a multi-target therapeutic candidate.

Although this study provides valuable mechanistic insights, experimental validation through in vitro and in vivo studies is required to confirm the predicted interactions and therapeutic efficacy of Rubiadin. Overall, the findings support Rubiadin as a promising natural compound for further investigation in breast cancer therapy.

Conclusion

The present study systematically elucidated the potential anticancer mechanisms of Rubiadin against breast cancer using an integrated network pharmacology and molecular docking approach. By identifying overlapping drug–disease targets and constructing a protein–protein interaction network, this study demonstrated that Rubiadin exerts its effects through the coordinated modulation of multiple molecular targets rather than a single pathway. Functional enrichment analysis highlighted the significant involvement of apoptosis, protein phosphorylation, and key oncogenic signaling pathways, particularly the PI3K-Akt signaling pathway, which plays a central role in breast cancer progression, survival, and therapeutic resistance. Molecular docking analysis further validated these findings by revealing favorable binding affinities of Rubiadin toward critical hub proteins such as ESR1, EGFR, MTOR, BCL2, and HSP90AA1, supporting its multi-target anticancer potential.

Despite these promising in silico findings, further experimental validation is essential to establish the therapeutic relevance of Rubiadin. Future studies should focus on in vitro investigations to evaluate its effects on breast cancer cell proliferation, apoptosis, and cell cycle regulation, followed by in vivo studies to assess its pharmacokinetic profile, bioavailability, safety, and antitumor efficacy. Additionally, exploring structural optimization and formulation strategies may enhance the biological activity and clinical applicability of Rubiadin. Overall, this study provides a strong theoretical foundation for the further development of Rubiadin as a potential natural therapeutic agent for breast cancer management and encourages its validation through experimental and clinical research.

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