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Original Article

Systems Pharmacology-Based *in silico* Decoding of the Polypharmacology Mechanisms of Silymarin against Breast Cancer

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ABSTRACT

Background: Breast cancer is a biologically heterogeneous disease driven by complex and interconnected molecular pathways, which often limit the effectiveness of conventional single-target therapies. This study applied an integrated systems pharmacology-based in silico approach to investigate the polypharmacological mechanisms of silymarin against breast cancer. **Materials and Methods:** Breast cancer-associated targets were systematically identified and analyzed through Protein-Protein Interaction (PPI) network analysis, hub gene analysis, and compound-target network mapping to identify key regulatory nodes. Network analysis revealed central hub genes involved in DNA damage repair, cell cycle regulation, apoptosis, hormone signaling, and oncogenic pathways. **Results:** Target prediction and overlap analysis demonstrated that major silymarin constituents interact with multiple breast cancer-associated molecular hubs, supporting a coordinated multi-target mechanism in critical cancer-related pathways, including p53, PI3K-AKT, Wnt/β-catenin signaling, and telomere maintenance. Molecular docking further validated stable, energetically favorable interactions between silymarin bioactive compounds and key cancer-driving proteins. *In silico* ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity profiling indicated acceptable pharmacokinetic properties and favorable safety profiles for the major silymarin constituents. **Conclusion:** Overall, this study provides systems-level evidence that silymarin exerts anticancer effects through multi-target and multi-pathway modulation, supporting its potential as a promising therapeutic candidate for breast cancer and warranting further experimental validation.

Keywords: ADME and Toxicity, Hub Gene, Molecular Docking, Network Pharmacology, Poly Pharmacology, Silymarin.

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INTRODUCTION

Breast cancer has been a major health issue of concern in the world and has been most commonly diagnosed among women across the world (Xiong *et al*., 2025). Although the modalities of diagnosis and therapy have improved, such as surgery, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy, intrinsic resistance, tumour recurrence, and metastatic progression often limit clinical outcome (Gupta *et al*., 2020; Ma., 2016). These problems are mainly due to the high molecular heterogeneity of breast cancer, which is motivated by intertwined signalling networks (Nascimento, 2020). Consequently, treatment approaches that manipulate more than one target simultaneously are in increasing demand to realize better and longer-lasting treatment outcomes (Odongo *et al*., 2021; Xulu *et al*., 2023). Natural products have been significantly considered in cancer research because their structural variety and polypharmacological characteristics (Koushki *et al*., 2023). In contrast to traditional single-target pharmaceuticals, most natural compounds may act on two or more molecular targets and signalling pathways and, therefore, are especially efficacious in complex diseases such as cancer. Notably, therapeutic resistance has also been overcome using natural products, which can alter oncogenic pathways and cancer populations by modulation cancer-stem (Saini *et al*., 2024; Ray *et al*., 2024).

Silymarin, a flavonolignan complex derived from *Silybum mariamum* (milk thistle), has been extensively used for its hepatoprotective effects and is well known for its antioxidant and anti-inflammatory properties (Ramasamy *et al*., 2008; Younas *et al*., 2018). In recent years, increasing evidence has highlighted its anticancer potential, particularly in breast cancer (Kim *et al*., 2021, and Alvarado Lozano *et al*., 2025). Experimental studies

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have shown that silibinin, a major active constituent of silymarin, can induce apoptosis and oncogenic microRNAs and inhibit key breast cancer drivers such as EGFR and HER2. Despite this promising observation, the comprehensive molecular mechanism underlying the anti-cancer activity of silymarin remains unclear (Binienda *et al.*, 2020; Delmas *et al.*, 2020).

The integration of systemic pharmacology with *in silico* approaches provides a powerful strategy for deciphering the complex mechanisms of multi-component natural products (Li *et al.*, 2017). By combining network pharmacology, molecular docking, and ADME toxicity prediction, this approach enables system-level analysis of drug target interactions, key signalling pathways, and pharmacokinetic behavior (Zhang *et al.*, 2024). These methodologies have been successfully applied to elucidate the molecular action of herbal compounds in breast cancer, revealing their involvement in pathways related to estrogen signalling, PI3K, AKT, MPAK, and apoptosis (Kurmi *et al.*, 2025).

In this study, we aimed to systematically investigate the polypharmacological mechanism of silymarin against breast cancer using an integrated *in silico* system pharmacology framework. By identifying key molecular targets, signalling pathways, and pharmacokinetic properties of silymarin, this study aims to provide insights that may support the development of silymarin as a multi-target therapeutic candidate for effective cancer management.

MATERIALS AND METHODS

Disease-Associated Target Identification

Breast cancer-associated genes were systematically collected from well-curated public databases to explore the polypharmacological mechanisms of silymarin (Bhat *et al.*, 2023). Gene Cards (GeneCards - Human Genes | Gene Database | Gene Search) were used to identify genes that have been reported to play a role in the pathogenesis of breast cancer (Basavarajappa *et al.*, 2023). The search strategy aimed to identify genes associated with major processes associated with cancer, such as cell proliferation, metastasis, apoptosis, and drug resistance (Azzahra *et al.*, 2024). Targets were selected based on their relevance scores and disease association strengths. The duplication of the entries was eliminated to make the datasets accurate, which led to the narrowing down of the list of unique breast cancer-related genes to a range of 50-300, which will be used to carry out further analyses (Zhang *et al.*, 2020).

Protein-Protein Interaction (PPI) Network Construction

The PPI network was developed using the STRING (Search Tool for the Retrieval of Interacting Gene /Protein) (STRING: functional protein association networks) database in Homo sapiens to examine the functional relationships among the identified disease targets (Laksmiani *et al.*, 2025). A threshold of high confidence (≥0.7) in interactions was selected to incorporate only trustworthy and interesting interactions. The obtained network was imported into Cytoscape software _v3.10.3, and further topologically analyzed to identify key patterns of interaction related to breast cancer biology.

Hub Gene Identification and Network Analysis

The PPI network was analyzed using Cytoscape for topological analysis to identify hub genes, which were nodes with a large degree of centrality (Sakle *et al.*, 2020), genes with high connectivity as regulators in the network (Vyas *et al.*, 2023). These hub genes were identified and provided some understanding of the major molecular control sites, which could be potential multivalent intervention sites for silymarin.

Compound Selection

The choice of compound narrowed down to the main bioactive compounds of Silymarin, i.e., Silypin, isosilypin, silydianin, and silychristin, (Wen *et al.*, 2007) since the compounds were reported to be pharmacologically relevant and have polypharmacological potential. The PubChem database was used to retrieve chemical structures and information on compounds (Bhat *et al.*, 2023; Ramzan *et al.*, 2025). Moreover, the reference drugs that had already proved their safety profile in the Drug Bank were effective to compare them, but the main focus was on silymarin constituents (Delmas *et al.*, 2020).

Target Prediction of Selected Compounds

The selection of compounds relied on a variety of *in silico* platforms used to predict the potential molecular targets of the selected compounds (silybin, isosilybin, silydianin, and silychristin) to increase the reliability of the predictions (Wen Z *et al.*, 2008). To determine probable target proteins with similar chemical properties, Swiss Target Prediction, the Similarity Ensemble Approach, and the STITCH database were used to predict potential protein targets from chemical interaction data and known interactions. The targets were predicted and compared with breast cancer-associated hub genes. The visualization of the overlapping targets through Venn diagram tools enabled the detection of common targets that mediate the anticancer activity of silymarin.

Compound-Target Network Construction

The network parameter degree centrality was determined in the interaction network of Cytoscape to determine the multivalent potential of each of the compound (Mokashi *et al.*, 2024). Compounds that were more connected, were regarded as having greater polypharmacology.

Functional Enrichment and Pathway Analysis

Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Gene and Genomes) pathway enrichment analyses were conducted

to determine the biological relevance of the discovered targets. Similar analyses revealed considerably enriched biological processes, molecular functions, cellular components, and signalling pathways of the reported predictions. The focus was on pathways pertinent to breast cancer progression and pathway crosstalk, providing a systems-level view of the mechanisms through which silymarin exerts its effects.

Molecular Docking Analysis

Interactions between the investigated silymarin compounds and the main breast cancer-associated hubs were tested using molecular docking (Purawarga Matada *et al.*, 2022). The RCSB Protein Data Bank (https://www.rcsb.org) provided protein structures, and docking was conducted using Auto Dock vina implemented through SwissDock Vina (SwissDock) (Ramzan *et al.*, 2025). Discovery Studio Visvalizer (BIOVIA Discovery Studio 2025) was used to analyse binding affinities and interaction profiles comprising hydrogen bonding and hydrophobic interactions. Compounds that showed positive binding energies with more than one target were regarded as potential opportunities for further research.

ADME and Toxicity Prediction

The selected silymarin constituents were screened for pharmacokinetic behavior and safety using established *in silico* ADME and toxicity prediction tools. The key pharmacokinetic parameters measured using ADME included drug-likeness, lipophilicity, gastrointestinal absorption, blood-brain barrier permeability, and compliance with the Lipinski rule of five. To evaluate safety, toxicity-related endpoints (including acute toxicity, and LD_{50}), hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity) were predicted using Tox21 and pkCSM. Compounds with poor pharmacokinetic properties or toxic liabilities forecasted through simple consideration were ruthlessly filtered out.

RESULTS

Identification of Breast Cancer-Associated Targets

A long list of molecular targets associated with breast cancer has been discovered Table 1, indicating the complicated and heterogeneous nature of the condition. These are mainly targets of DNA damage and repair, cell cycle regulation, apoptosis, oncogenic signalling, hormone responsiveness, angiogenesis, and epigenetic regulation.

Protein- protein interaction network analysis

The Protein-Protein Interaction (PPI) network of breast cancer-associated targets is shown in Figure 1. The network was densely interconnected, indicating strong functional coordination among the identified proteins.

Hub Gene Identification and Network Analysis

As illustrated in Figure 2, hub gene analysis of the protein interactions revealed a subset of highly connected nodes occupying central positions within the networks. Genes such as TP53, PTEN, BRCA1, ATM, MYC, KRASM AKT1, PIK3CA, EGFR, ERBB2, ESR1, CCND1, CTNNB1, BCL2, and TERT are

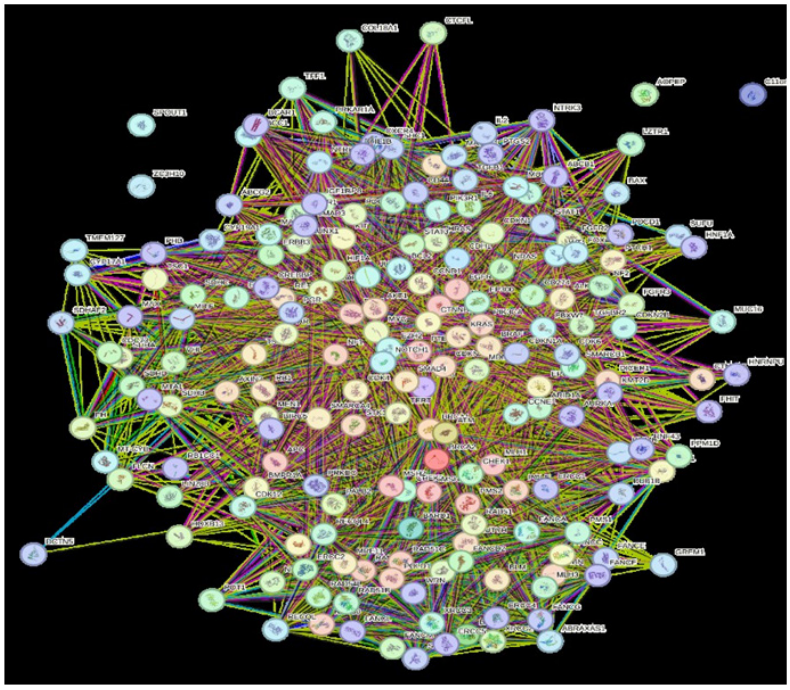

Figure 1: Protein–Protein Interaction Map representing key signalling and regulatory proteins involved in breast cancer signalling pathways.

Table 1: Breast Cancer–Associated Molecular Targets. List of genes implicated in breast cancer pathogenesis.

Disease	Gene
Breast Cancer	BRCA2, BRCA1, ATM, PALB2, BRIP1, CHEK2, BARD1, CDH1, C11orf65, TP53, MSH6, MSH2, MLH1, ERBB2, EGFR, PTEN, LOC126862571, APC, PMS2, RAD51D, RAD51C, RAD_{50}, RAD51L3-RFFL, NF1, ESR1, POLD1, PIK3CA, POLE, NBN, STK11, DICER1, CDKN2A, CTNNA1, AKT1, MUTYH, AXIN2, RET, MET, BRAF, KRAS, SMAD4, RB1, BLM, TSC2, SMARCA4, ALK, MRE11, CDK4, PTCH1, EPCAM, MSH3, CTNNB1, BAP1, KIT, FANCC, BMPR1A, CCND1, TSC1, TERT, MYC, MEN1, FGFR2, AR, RAD51, SDHA, MTOR, PDGFRA, CDKN1B, FH, SDHB, HRAS, ATR, MDM2, RUNX1, HIF1A, HOXB13, XRCC2, FGFR3, NTHL1, FLCN, H19, TGFBR2, VHL, FANCM, CASP8, STAT3, FGFR1, AOPEP, CDC73, NRAS, ERBB3, SDHC, FANCD2, SDHD, ERCC2, MIR21, NF2, PPM1D, PVT1, CD274, CHEK1, IL6, POT1, ARID1A, RAD54L, LZTR1, MLH3, RAD51B, SRC, ERBB4, RECQL4, PMS1, EP300, GAS5, GATA3, TFF1, SMARCB1, VEGFA, PRKAR1A, CCNE1, XRCC3, FANCA, CDK12, UCA1, MUC16, CDKN2B, LOC129390903, PIK3R1, MAP2K1, MIR125A, LOC111589215, JUN, MALAT1, EZH2, BCL2, MIR145, FBXW7, ABCC1, MT-CYB, MUC1, PCAT1, WT1, NOTCH1, CYP17A1, TMEM127, FOXA1, RECQL, MIR143, TUG1, CDKN2B-AS1, BAX, BCAR4, NTRK1, ABRAXAS1, MIR221, SNHG1, NEAT1, MIR17, MIR155, CYP19A1, GREM1, MAP3K1, MIR30A, HOTAIR, SUFU, TGFB1, HMMR, MIR205, MIR141, MIR146A, MIR200C, STAT1, CDK6, SDHAF2, BUB1B, MITF, IL2, PGR, MIR126, MIR27A, MIR182, HULC, SHC1, FANCE, MIR195, ABCG2, LUCAT1, MIR31, PRKDC, MIR222, SLX4, DCTN5, CDKN1A, CD44, FANCG, PTGS2, WRN, RNF43, KMT2D, MIR146B, CASC9, SNHG7, MIR200B, CASC2, CXCR4, SMAD3, FANCF, CASC15, SNHG16, MIR10B, MIR20A, $_{MAX}$, IL1B, BIRC5, MIR210, IGF1R, MEG3, AFAP1-AS1, CREBBP, FANCL, MIR18A, XIST, MIR29C, DANCR, OIP5-AS1, NTRK3, PRNCR1, MIR200A, ERCC5, AURKA, PHB1, ABCB1, PDCD1, RB1CC1, MIR335, ERCC4, FHIT, SNHG12, MTA1, HOTAIRM1, HNF1A, BCAR1, MIR127, CYTOR, ERCC1

indicators of the critical roles they play in the cancer-associated processes in the breast.

Target Prediction and Overlap Analysis

Target-prediction and overlap analyses Table 2 and Figure 3, revealed a partial overlap of breast cancer-associated with silymarin-predicted targets. There were 15 disease-specific targets and 56 silymarin-specific targets, with five overlapping genes found in both datasets. These common homologous targets encompass key regulators of the cell cycle, apoptosis, oncogenic signalling, and tumor suppression, implying that silymarin can directly regulate key molecular nodes associated with breast cancer progression. The existence of other silymarin-specific targets also suggests the ability to respond to other auxiliary pathways related to metabolism, oxidative stress and signal transduction.

Compound Target Network Analysis

The number of hits of the major silymarin constituents, silybin, isosilybin, siluydianin, and silychristin, targeting several cancer-specific early warning indicators, such as CTNNB1, TERT, TP53, BCL2, and CCND1, are shown Figure 4.

KEGG Pathway Enrichment Analysis

KEGG pathway mapping also proves that the hub targets are centrally located in numerous oncogenic pathways related to cancer progression (Figures 5A-5D). The central targets associated with prostate, colorectal, and gastric cancer responses were plotted onto these pathways as shown in Figures 5A-5C, which were associated with cell cycle regulation, apoptosis, Wnt / -catenin signalling, PI3K- AKT signalling, and p53-mediated DNA-damage response. Most interestingly, key nodes such as TP53, CTNNB1, CCND1, BCL2 and TERT always serve as convergence points of the pathways indicating their regulatory role in specific types of cancer. Quantitative enrichment analysis Figure 5D) further confirmed the significant over-representation of cancer-associated pathways, including colorectal, prostate, gastric and breast cancer, along with the p53 signaling pathway.

Gene Ontology Enrichment Analysis

The hub genes identified by Gene Ontology enrichment analysis revealed that functionally clustered around core regulatory mechanisms driving breast cancer progression Figures 6A-6C. Molecular function enrichment analysis Figure 6A revealed that DNA-dependent DNA annealing activity, histone deacetylase regulation, transcription factor binding, and protein kinase binding were the most enriched, highlighting the importance of epigenetic modulation and transcriptional control. Cellular component analysis Figure 6B showed significant enrichment of β-catenin-TCF transcriptional complexes, telomere cap complexes, transcription repressor complexes, and adherens junctions, underscoring coordinated regulation of Wnt signaling, telomere integrity, and cell-cell adhesion. Biological process enrichment Figure 6C was dominated by telomere maintenance of telomerase, cell cycle transition regulation, G1/S, apoptotic signalling, and mitotic control of the cell cycle.

Molecular Docking Analysis

Molecular docking verified the binding capacity of the main silymarin components (silybin, isosilybin, silydianin, and

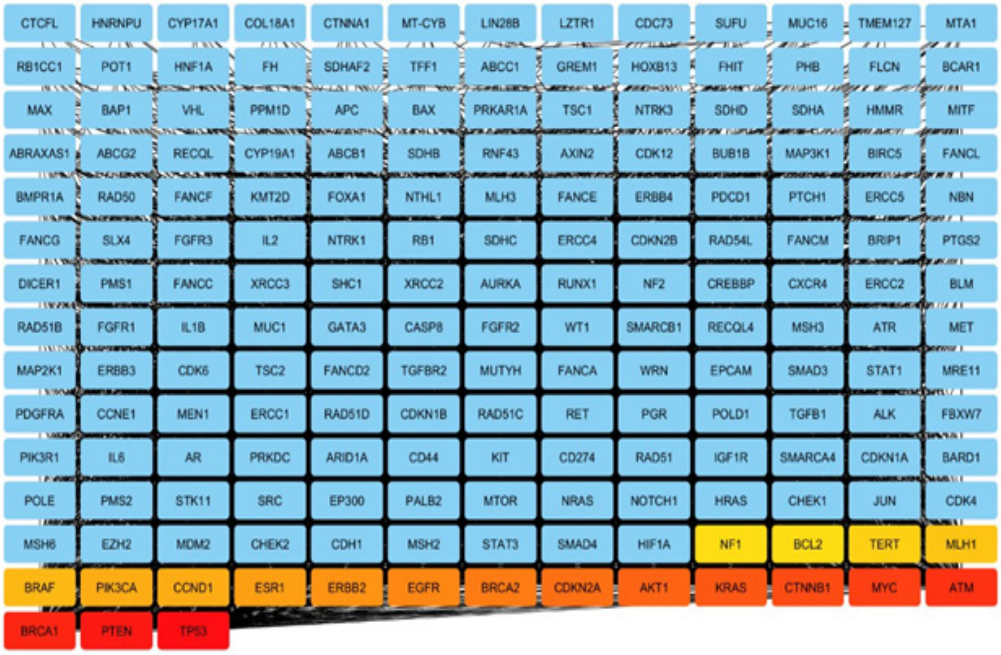

Figure 2: Identification and functional characterization of Hub Genes in the breast cancer PPI network.

Table 2: Target Prediction and Overlap Analysis between Breast Cancer and Silymarin Targets.

Disease Target	Silymarin Target
PTEN, BRCA1, ATM, MYC, KRAS, AKT1, CDKN2A, BRCA2, EGFR, ERBB2, ESR1, PIK3CA, BRAF, MLH1, NF1	IKBKB, TXN, TXN2, CA7, CA2, CA3, CA1, CBS, CYP1B1, AMACR, ABCC2, TAS2R31, CA6, CA12, CA13, CA5B, ABCB1, CA5A, MMP9, IGFBP3, TP53, SNAI1, CTNNB1, DNMT1, MMP2, CCND1, VEGFA, RAF1, MMP13, MMP12, KLK1 KLK2, CYP1B1, TAS2R31, CYP19A1, RXRA, KIT, FGFR1, ESR2, PTGS1, BCL2, ABCG2, ABCC1, SHBG, CBR1, MAPT, TERT, PGD, ST3GAL3, FUT7, FUT4, STAT1, SQLE, MAOB, MMP2, MT-ND4, KDR, KCNH2, GABRA1 GABRB2 GABRG2 PLA2G1B, SRC, MET, FFAR1, PTAFR, PGD

silychristin) to the primary breast cancer hubs targets, such as CTNNB1, TERT, BCL2, CCND1, and TP53 Figures 7-10. The binding affinities were favorable for all compounds, facilitating the formation of ligand–protein complexes Table 3. Among them, silydianin exhibited the greatest interaction with TERT (−8.650 kcal/mol-1), indicating its high potential to interfere with the cellular process of immortality through telomerase. Silybin and silychristin consistently bind to several targets, especially BCL2 and TP53 and could potentially regulate apoptosis and tumor-suppressor signalling.

ADME and Toxicity Profiling

In silico ADME and toxicity analyses were performed to evaluate the pharmacokinetic suitability and safety profiles of the major silymarin constituents (silybin, isosilybin, silydianin, and silychristin) Tables 4 and 5. All compounds exhibited comparable molecular weights and acceptable lipophilicity (LogP 1.97-3.10), with no Lipinski rule violations except for silychristin, which showed a single violation due to an elevated polar surface area. The predicted bioavailability score (0.55) was uniform across all compounds, indicating moderate oral drug—likeness, although gastrointestinal absorption was predicted to be low and blood-brain barrier permeability was absent, suggesting peripheral selectivity. Toxicity prediction revealed favorable safety profiles, with silybin, isosilybin, and silychristin showing predicted (LD_{50} values of 10,000 mg/kg). Notably, none of the compounds were predicted to possess hepatotoxic, carcinogenic, mutagenic or cytotoxic liabilities. Overall, these results support the tolerable pharmacokinetic behavior and safety profile of silymarin constituents, indicating their appropriateness for use in future experiments as multiple-target anticancer agents.

DISCUSSION

The role of genomic instability in the development of breast cancer can be demonstrated by the prominent expression of DNA repair and tumor suppressor genes, including BRCA1, BRCA2, ATM, TP53, and PTEN, whereas the significance of major signalling pathways such as PI3K-AKT is shown by oncogenic drivers, such as MYC, KRAS, BRAF, AKT1, ERBB2, EGFR, and OIKEYA. The presence of hormone-related genes

Table 3: Docking scores of silymarin constituents against selected breast cancer hub targets.

Compound name	Target name	PDB id	Docking score
Silybin	CTNNB1	1JDH	-7.251
	TERT	2NQ6	-8.037
	BCL2	5UUP	-7.540
	CCND1	6P8E	-6.748
	TP53	8SWJ	-7.207
Isosilybin	CTNNB1	1JDH	-6.795
	TERT	2NQ6	-7.549
	BCL2	5UUP	-7.661
	CCND1	6P8E	-6.471
	TP53	8SWJ	-6.312
Silydianin	CTNNB1	1JDH	-6.781
	TERT	2NQ6	-8.650
	BCL2	5UUP	-7.270
	CCND1	6P8E	-7.036
	TP53	8SWJ	-7.063
Silychristin	CTNNB1	1JDH	-6.404
	TERT	2NQ6	-7.553
	BCL2	5UUP	-6.633
	CCND1	6P8E	-7.537
	TP53	8SWJ	-6.810

(ESR1, AR, PGR) and regulators of angiogenesis and metastasis (VEGFA, CXCR4, STAT3) provides additional evidence for the multifaceted molecular basis of breast cancer. In general, these findings indicate that breast cancer is controlled by a highly interdependent multivalent network, which has a solid base for further system pharmacology and polypharmacology studies. The enrichment in PPI interaction, with nodes indicating a high level of connectivity, suggests the presence of central regulatory functions in DNA repair, cell cycle regulation, apoptosis, and oncogenic signalling processes vital to the advancement of breast cancer. This complex interaction pattern highlights the molecular complexity of this disease and justifies the need for a systems-level multi-target therapeutic approach.

The nodes occupy a central position, which plays a significant role in disease development and progression, as these hub genes are mostly engaged in DNA damage response, cell cycle, apoptosis regulation, and oncogenic signalling pathways. The identification of these central regulators also enhances their applicability as major molecular targets and provides a strong basis for further investigations of compound-target interactions and molecular-docking studies. All target prediction overlap analysis results support the polypharmacological profile of silymarin and confirm its applicability as a multi-target approach for the treatment of breast cancer. The multi-target nature of silymarin engagement with cell cycle control-related targets,

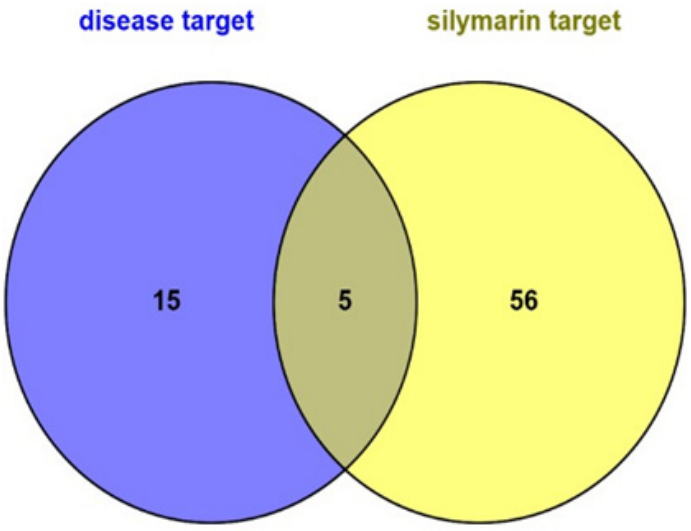

Figure 3: *In silico* identification of potential therapeutic targets of Silymarin and their overlap with breast cancer-related genes.

apoptosis, telomere repair, and oncogenic signalling implies that silymarin has coordinated regulatory actions on key pathways that support cancer development. In general, this network helps sustain a systems-level process according to which silymarin can adjust various molecular nodes instead of targeting only one.

In the KEGG pathway, multiple tumor-specific and shared signaling pathways support a systems-level, multi-pathway

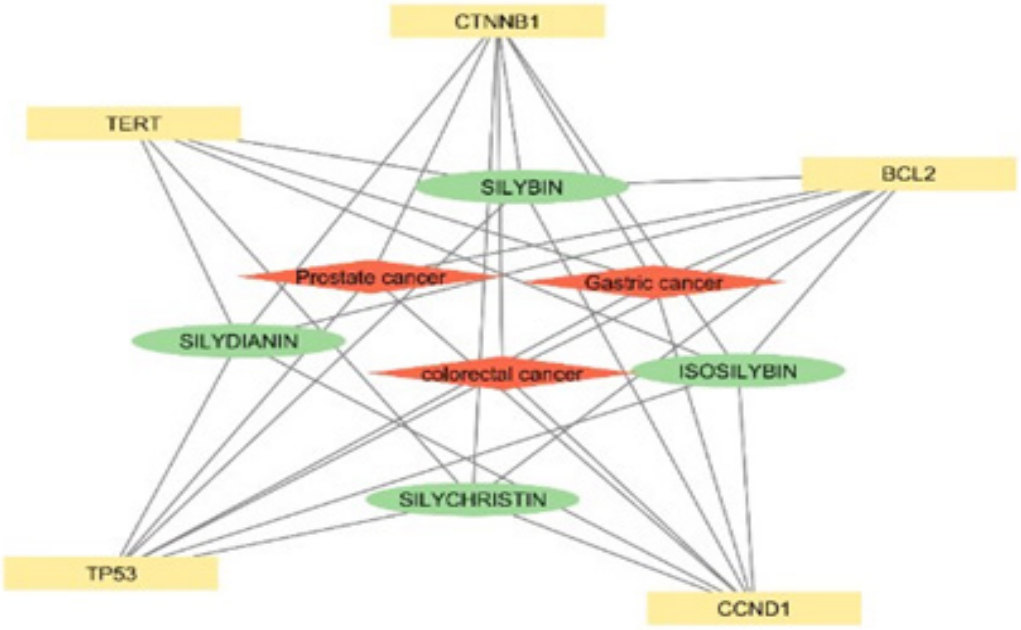

Figure 4: Ligand–Target Interaction Network of Bioactive Constituents from Silymarin.

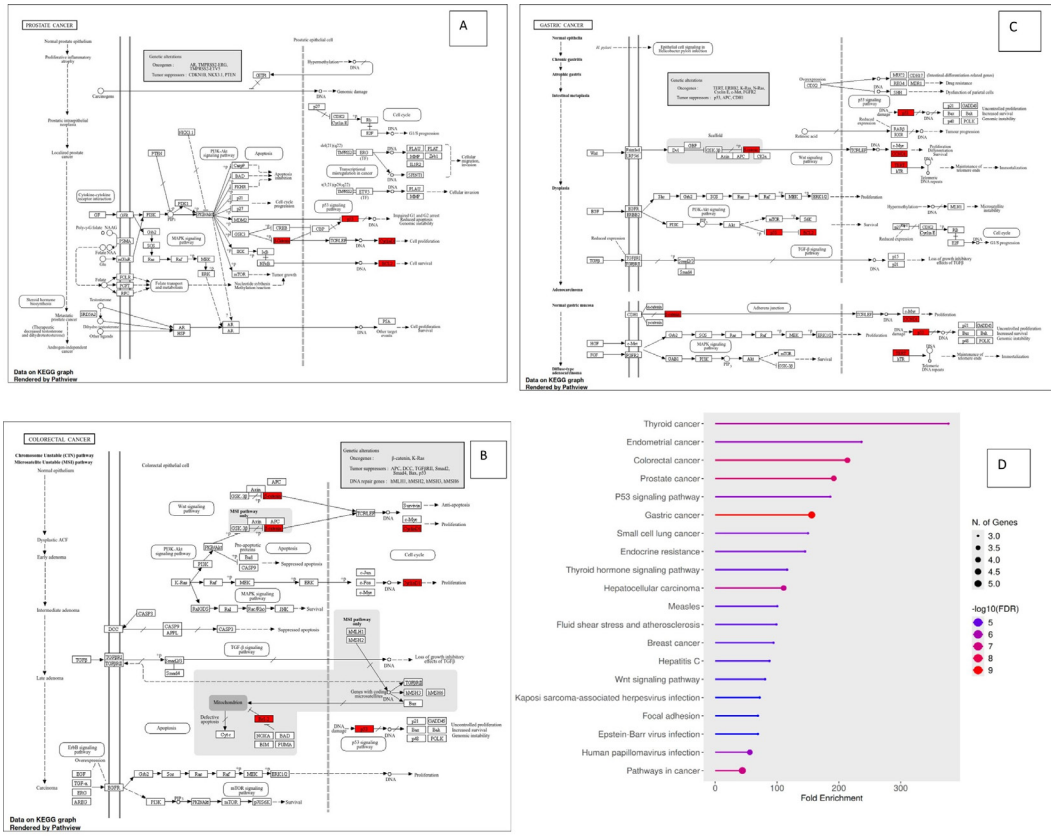

Figure 5: KEGG pathway enrichment and pathway mapping of identified hub targets. (A, B and C) KEGG pathway maps for prostate, colorectal and gastric cancer, highlighting key. target involvement. (D) Dot plot showing significantly enriched KEGG pathways based on fold enrichment and FDR.

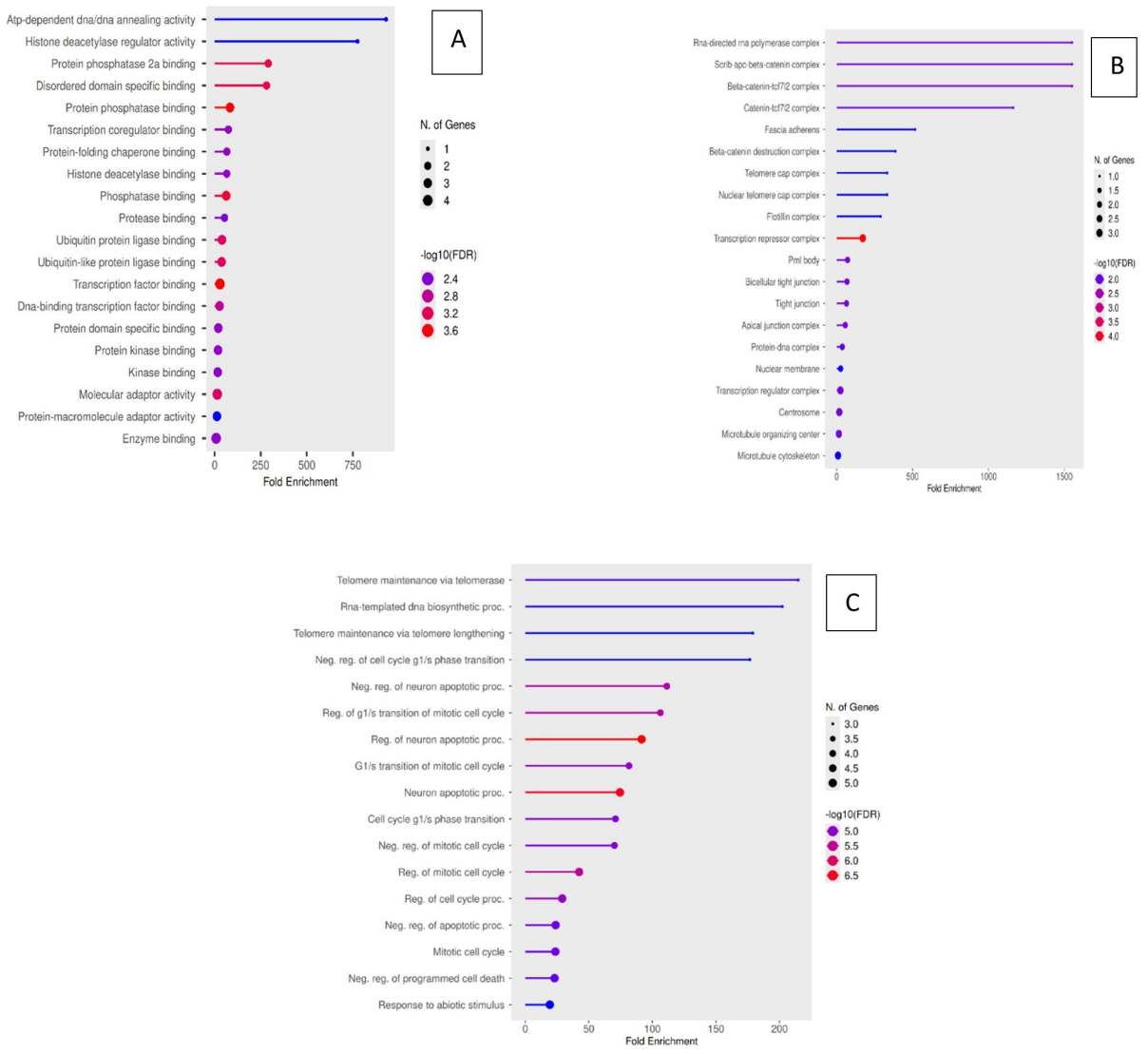

Figure 6: Gene Ontology enrichment analysis of hub genes showing significantly enriched (A) Molecular functions, (B) Cellular components, and (C) Biological processes relevant to breast cancer.

Table 4: Predicted ADME properties of silymarin bioactive compounds.

Parameter	Silybin	Isosilybin	Silydianin	Silychristin
Molecular Weight (g/mol)	482.44	482.44	482.44	482.44
LogP	2.36	3.10	1.97	2.31
H-Bond Donors	5	5	5	6
H-Bond Acceptors	10	10	10	10
TPSA (Å²)	155.14	155.14	162.98	166.14
Lipinski Rule Violations	0	0	0	Yes; 1 violation
Bioavailability Score	0.55	0.55	0.55	0.55
GI Absorption	LOW	LOW	LOW	LOW
BBB	NO	NO	NO	NO
Result Summary	Drug-like	Drug-like	Drug-like	Drug-like

regulatory mechanism, reinforcing the polypharmacological relevance of the identified targets in cancer. Gene ontology enrichment analysis data indicated that the hub genes converge on the pathways of genomic stability, proliferation, and apoptosis, which proves a systems-level, multi-target mechanism of the anti-cancer efficacy of silymarin constituents. The molecular docking findings of interaction analyses indicated some stable hydrogen bonds, π-stacking, and hydrophobic contacts in

Table 5: Predicted ADME toxicity profiles of silymarin bioactive compounds.

Parameter	Silybin	Isosilybin	Silydianin	Silychristin
LD_{50} (mg/kg)	Predicted LD_{50}: 2000 mg/kg	Predicted LD_{50}: 2000 mg/kg	Predicted LD_{50}: 10000 mg/kg	Predicted LD_{50}: 2000 mg/kg
Predicted Toxicity Class				
Hepatotoxicity				
Carcinogenicity				
Mutagenicity				
Cytotoxicity				
Safety Profile	Safe	Safe	Safe	Safe

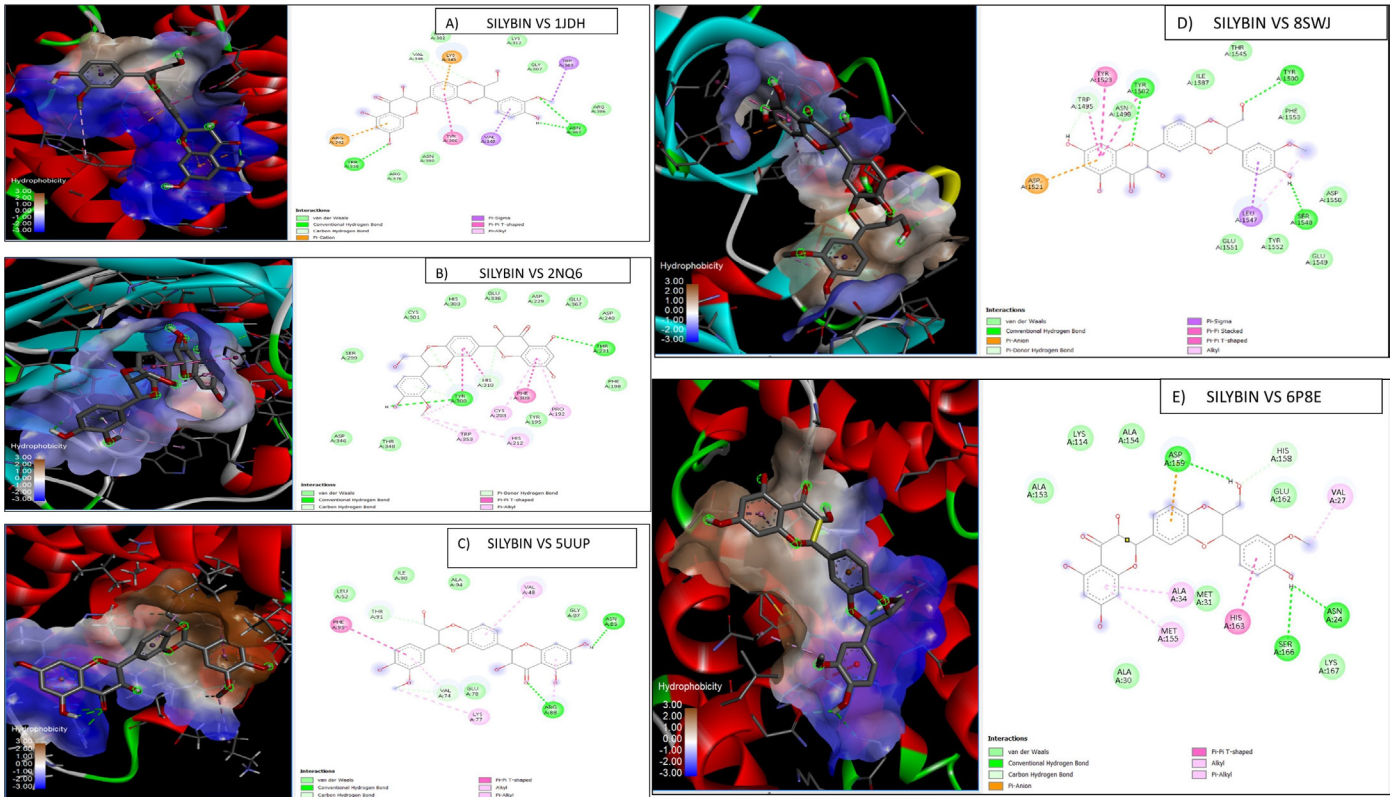

Figure 7: Molecular docking interactions of silymarin bioactive compounds (SILYBIN A, B, C, D and E) with key breast cancer hub proteins.

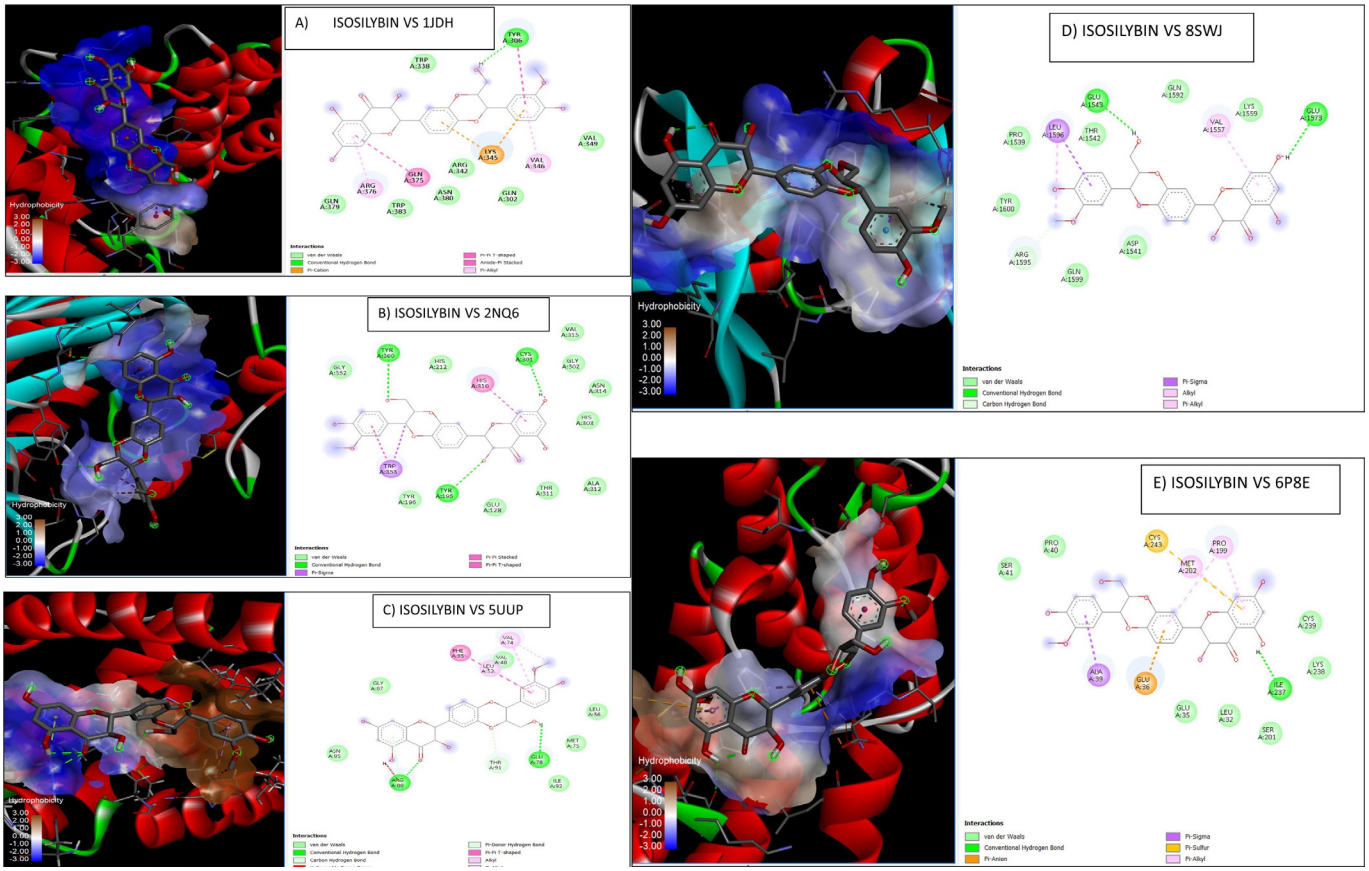

Figure 8: Molecular docking interactions of silymarin bioactive compounds (ISOSILYBIN A, B, C, D and E) with key breast cancer hub proteins.

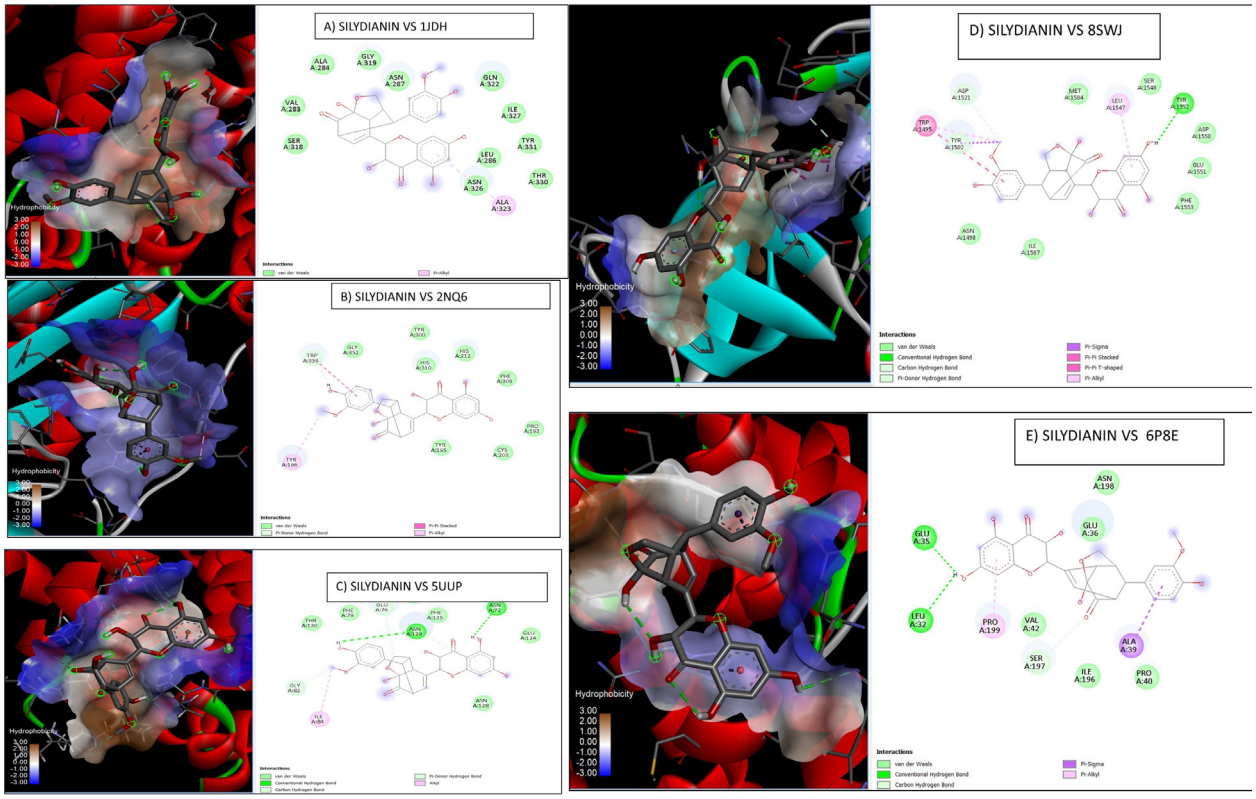

Figure 9: Molecular docking interactions of silymarin bioactive compounds (SILYDIANIN A, B, C, D and E) with key breast cancer hub proteins.

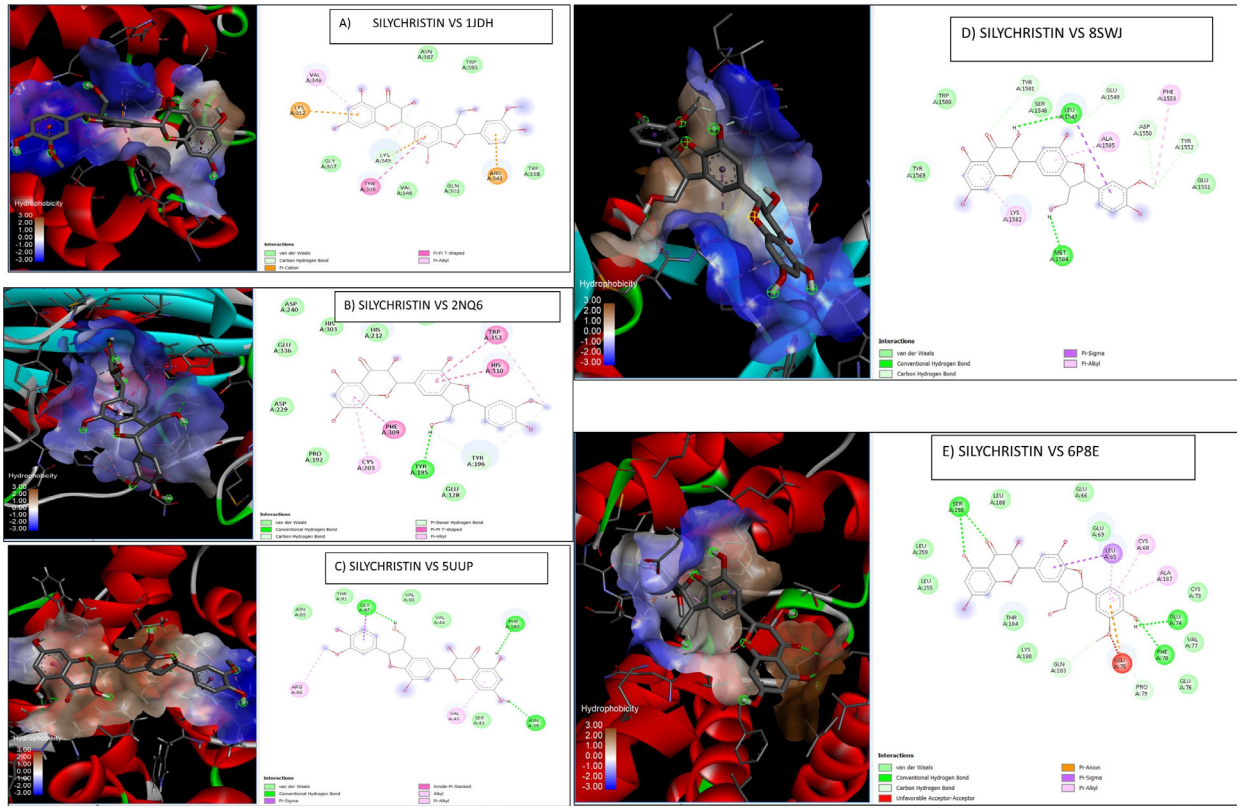

Figure 10: Molecular docking interactions of silymarin bioactive compounds (SILYCHRISTIN A, B, C, D and E) with key breast cancer hub proteins.

the active binding pockets, which demonstrated an optimal physical complication between the ligand and the active site. Moderate yet consistent affinities toward CTNNB1 and CCND1 further indicate the possible suppression of Wnt/β-Catenin signalling and cell cycle progression. Collectively, these docking results corroborate the network-based findings and highlight the multi-target, polypharmacological anticancer potential of silymarin at the molecular level.

TP53 is a critical tumor suppressor gene frequently mutated in breast cancer and plays a central role in DNA damage response and apoptosis regulation. The identification of TP53 as a hub gene in the present network analysis is consistent with previous studies demonstrating its pivotal role in tumor suppression and therapeutic response in breast cancer (Xiong *et al.*, 2025). Although the present study provides valuable insights into the polypharmacological mechanisms of silymarin against breast cancer using integrated *in silico* approaches, several limitations should be acknowledged. The findings of this study are primarily based on computational predictions, including network pharmacology, molecular docking, and ADME–toxicity profiling. Therefore, the predicted compound–target interactions and signaling pathways require further validation through experimental studies. Future investigations involving *in vitro* cell-based assays, molecular biology experiments, and *in vivo* animal models will be essential to confirm the therapeutic potential of silymarin and to validate the molecular mechanisms proposed in this study.

CONCLUSION

This paper employed an in-silico approach involving an integrated systems-pharmacology tool to clarify the multi-target anticancer effects of silymarin in breast cancer. Molecular interaction Network pharmacology and protein-protein interaction studies showed that there exists a highly connected molecular network that was comprised of key regulatory nodes such as TP53, BRCA1, BRCA2, CTNNB1, MYC, TERT, BCL2, CCND1, KRAS, and AKT1 controlling genomic stability, cell-cycle regulation, apoptosis, and oncogenic signalling. Target overlap and compound-target network analyses showed that the silymarin components act on multiple of these key nodes, which indicates that a network-based polypharmacological mode of action occurs.

Functional enrichment studies revealed that silymarin-linked targets play an important role in cancer-related pathways, especially p53, PI3KAKT, and Wnt/ -catenin transduction, as well as telomere maintenance and transcriptional regulation. Molecular docking also supported these results by demonstrating stable and energetically favourable reactions of silymarin bioactive compounds TERT with silydianin and TP53 and BCL2 with silybin. The predictions of ADME and toxicity showed that the first-order silymarin constituents exhibit acceptable

pharmacokinetic and positively favourable safety profiles. Together, these findings demonstrate strong systems-level evidences present in favour of silymarin as a viable multi-target therapeutic candidate for breast cancer and, therefore, need to be further experimentally and preclinically validated.

ABBREVIATIONS

P53: Tumor protein p53; **PI3K, AKT, MAPK:** Phosphoinositide 3-kinase, Protein kinase B, Mitogen-activated protein kinase; **Wnt/β-catenin:** Wingless/Integrated–beta-catenin signaling pathway; **ADME:** Absorption, Distribution, Metabolism, Excretion; **EGFR:** Epidermal Growth Factor Receptor; **HER2:** Human Epidermal Growth Factor Receptor 2; **PPI:** Protein-protein Interaction; **STRING:** Search Tool for the Retrieval of Interacting Genes/Proteins; **STITCH:** Search Tool for Interactions of Chemicals; **KEGG:** Kyoto Encyclopedia of Genes and Genomes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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