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Original Article

Potential molecular targets and pathways of *Zingiber officinale* in estrogen receptor-positive breast cancer: An integrated network pharmacology and molecular docking study

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Abstract

Breast cancer remains a major global health burden, and the multi-target anti-cancer mechanisms of *Zingiber officinale* against estrogen receptor-positive (ER+) breast cancer require further clarification. This study employed an integrative approach combining network pharmacology and molecular docking to systematically investigate the anti-breast cancer mechanisms of *Z. officinale*. Ginger rhizome ethanol extract was profiled using LC–MS/MS to identify bioactive compounds. Putative compound targets were predicted using PubChem and SwissTargetPrediction, while ER+ breast cancer-related genes were retrieved from GeneCards. Overlapping targets were identified and subjected to GO/KEGG enrichment analysis, followed by protein-protein interaction (PPI) network construction using STRING, network visualization in Cytoscape, and hub-gene identification using CytoHubba. Molecular docking was then performed using CB-Dock2/AutoDock Vina against key hub proteins, with tamoxifen, doxorubicin, and 5-fluorouracil used as reference drugs. Nine bioactive compounds were identified. Target prediction identified 445 potential protein targets for ginger compounds and 10,003 ER+ breast cancer-related genes, with Venn diagram analysis revealing 266 overlapping targets representing therapeutic intervention points. KEGG pathway enrichment identified 20 significantly enriched pathways (false discovery rate less than 0.05), including EGFR tyrosine kinase inhibitor resistance, estrogen signaling, PI3K-Akt signaling, and MAPK signaling pathways. PPI network analysis identified ten hub genes: *AKT1, GAPDH, SRC, STAT3, EGFR, ESR1, PPARG, MAPK3, MMP9*, and *TLR4*. Adenosine demonstrated exceptional affinity to GAPDH (–9.0 kcal/mol), approaching that of tamoxifen (–8.9 kcal/mol). 6-Gingerol demonstrated strong binding to MMP9 (–7.9 kcal/mol), ESR1 (–7.6 kcal/mol), and MAPK3 (–7.6 kcal/mol). Detailed interaction analysis revealed that the adenosine-GAPDH complex formed multiple hydrogen bonds with THR65, ARG15, GLU17, THR87, and ASN239, with extensive hydrophobic contacts stabilizing the complex within the glycolytic active site. These findings provide mechanistic insights supporting ginger's therapeutic potential as a complementary natural product in ER-positive breast cancer management and identify specific molecular targets for future preclinical and clinical investigations.

Keywords: Breast cancer, ginger, *Zingiber officinale,* molecular docking, network pharmacology

Introduction

Breast cancer remains the most prevalent malignancy among women globally, representing a critical public health challenge with substantial morbidity and mortality [1,2]. According to the

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latest Global Cancer Observatory (GLOBOCAN) data, approximately 2.3 million new breast cancer cases and 670,000 deaths occurred worldwide in 2022, with projections indicating a 38% increase in incidence and a 68% rise in mortality by 2050, disproportionately affecting low- and middle-income countries [1,3]. On average, 1 in 20 women worldwide will be diagnosed with breast cancer in their lifetime, with incidence rates varying substantially across geographic regions—from approximately 100 per 100,000 women in Australia and New Zealand to 27 per 100,000 in South-Central Asia [1]. Estrogen receptor-positive (ER+) breast cancer constitutes 70-80% of all breast cancer cases and is characterized by distinct molecular pathways amenable to targeted therapeutic interventions [4]. Despite significant advances in early detection and systemic therapies, breast cancer mortality rates have declined by 44% in high-income countries since 1989. Yet, substantial disparities persist, with mortality rates remaining unchanged in some populations, underscoring the urgent need for novel therapeutic approaches [2].

Current treatment modalities for breast cancer encompass surgery, radiation therapy, chemotherapy, hormonal therapy, and targeted biological agents. However, these conventional treatments are frequently associated with significant adverse effects, high costs, and the emergence of drug resistance, particularly in hormone receptor-positive and triple-negative breast cancer subtypes [4,5]. Chemotherapeutic agents such as doxorubicin and 5-fluorouracil, while effective, often induce severe toxicities including cardiotoxicity, myelosuppression, and gastrointestinal disturbances [6]. Moreover, endocrine resistance develops in approximately 30–40% of patients receiving hormonal therapy, limiting long-term treatment efficacy [7]. These limitations have increased interest in complementary and alternative medicine, particularly natural product-based therapeutics, which may offer multi-targeted mechanisms of action with potentially improved safety profiles.

Ginger (*Zingiber officinale*), a perennial herbaceous plant belonging to the Zingiberaceae family, has been utilized for millennia in traditional medicine systems across Asia, Africa, and the Middle East for its anti-inflammatory, antioxidant, and gastrointestinal protective properties [8]. The rhizome contains a rich array of bioactive phytochemicals, including gingerols, shogaols, paradols, zingerone, and various terpenoids, which collectively contribute to its pharmacological activities [9]. Recent preclinical investigations have demonstrated that ginger constituents exhibit potent anti-cancer properties through multiple mechanisms, including apoptosis induction, cell cycle arrest, anti-metastatic activity, anti-angiogenesis, and modulation of oxidative stress [8,10]. Specifically, 6-gingerol, the predominant bioactive component, has been shown to suppress proliferation and colony formation in breast cancer cell lines MCF-7 and MDA-MB-231 through upregulation of pro-apoptotic proteins (Bax) and downregulation of anti-apoptotic proteins (Bcl-2), while sparing normal mammary epithelial cells [11]. Additionally, ginger-derived compounds have demonstrated efficacy in reducing chemotherapy-induced nausea and vomiting, suggesting potential as both therapeutic and supportive agents in cancer treatment [12,13]. Despite these promising findings, the precise molecular mechanisms underlying ginger's anti-breast cancer activity remain incompletely elucidated, particularly regarding its multi-targeted effects on complex signaling networks.

Traditional drug discovery paradigms, predicated on the "one drug, one target, one disease" model, have proven increasingly inadequate for addressing complex multifactorial diseases such as cancer, which involve perturbations across multiple interconnected molecular pathways [14,15]. Network pharmacology has emerged as a systems biology-based approach that integrates computational biology, bioinformatics, and high-throughput omics technologies to elucidate the complex interactions among drugs, targets, and diseases through the construction and analysis of multi-layered biological networks [14,16]. Unlike conventional single-target methodologies, network pharmacology embraces the principle of polypharmacology, recognizing that therapeutic efficacy often arises from modulation of multiple protein targets within disease-associated networks rather than high selectivity for individual molecules [15,17]. This paradigm shift is particularly relevant for natural products, which characteristically contain multiple bioactive constituents capable of simultaneously modulating diverse biological pathways—a phenomenon termed "multi-component, multi-target" activity [18]. Network pharmacology facilitates the systematic prediction of compound-target interactions, identification of hub targets within protein-protein interaction networks, elucidation of key biological pathways, and ultimately, the

mechanistic understanding of therapeutic actions [14,16]. Furthermore, molecular docking, a structure-based computational technique, complements network pharmacology by predicting the binding modes and affinities of ligands to protein targets, thereby providing atomic-level insights into drug-target interactions and validating predicted therapeutic targets [19,20].

The integration of network pharmacology and molecular docking represents a synergistic approach for investigating the anti-cancer mechanisms of complex natural products. This computational strategy enables the systematic identification of bioactive compounds, prediction of their molecular targets, mapping of target interactions within biological networks, and validation of binding interactions [21]. Such integrative methodologies have been successfully applied to elucidate the therapeutic mechanisms of various traditional medicines in cancer treatment, demonstrating their utility in accelerating drug discovery and mechanistic research [14,18]. However, to date, comprehensive network pharmacology studies specifically investigating ginger's multi-targeted mechanisms against ER-positive breast cancer remain limited, and the molecular determinants of ginger compound-protein interactions have not been systematically characterized.

The aim of this study was therefore to elucidate the anti-breast cancer mechanisms of *Z. officinale* through an integrated approach combining network pharmacology and molecular docking. Specifically, the study sought to: (1) identify bioactive compounds from ginger and predict their potential protein targets; (2) construct compound-target-disease networks to identify overlapping targets between ginger compounds and ER-positive breast cancer; (3) perform pathway enrichment analysis to elucidate the biological processes and signaling pathways modulated by ginger; (4) identify hub genes through protein-protein interaction network analysis; and (5) validate the predicted targets through molecular docking to assess binding affinities and characterize key intermolecular interactions. The findings from this investigation will provide mechanistic insights into ginger's multi-targeted anti-breast cancer activity, identify key molecular targets for experimental validation, and contribute to the scientific foundation for developing ginger-based complementary therapeutics or chemopreventive strategies in breast cancer management.

Methods

Preparation of ginger extracts

Ginger (*Z. officinale*) was obtained from Medan, authenticated and identified by the Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. Rhizomes of ginger were cleaned with distilled water, thinly sliced, and dried in an oven at 50°C for 24 h. Dried slices of ginger were further reduced in size using a blender to obtain dry simplicia powder. Next, the simplicia underwent maceration with 1000 g of dry simplicia powder in 10 L of 70% ethanol for three days, with occasional stirring at room temperature (25°C). The filtrate was separated, and maceration was repeated in triplicate. The combined filtrates were concentrated using a rotary evaporator at 70°C to produce a thick extract of ginger, which was stored in aluminium foil for further testing. This extraction method refers to a similar published study [22].

Compound identification of ginger extract using LC-MS/MS

In this study, an untargeted metabolomics analysis was carried out utilizing liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The experiment employed a Waters 2D UPLC (Waters, USA) in conjunction with a Q-Exactive HF high-resolution mass spectrometer (Thermo Fisher Scientific, USA) for metabolite separation and detection. This instrument was operated in both positive-ion and negative-ion modes to enhance metabolite coverage.

For chromatographic conditions, the analysis was conducted using a Waters 2D UPLC (Waters, USA) linked to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, USA) equipped with a heated electrospray ionization (HESI) source and managed by the Xcalibur 2.3 software program (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was achieved on a Waters ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 mm × 100 mm, Waters, USA), with the column temperature kept at 45°C. The mobile phase was composed of 0.1% formic

acid (A) and acetonitrile (B) in the positive mode, while in the negative mode, it was composed of 10 mM ammonium formate (A) and acetonitrile (B). The gradient conditions were set as follows: 0–1 min, 2% B; 1–9 min, 2%–98% B; 9–12 min, 98% B; 12–12.1 min, 98% B to 2% B; and 12.1–15min, 2% B. The flow rate was maintained at 0.35 mL/min, and the injection volume was set at 5 µL.

For mass spectrometry conditions, the settings for positive/negative ionization modes were as follows: spray voltage at +3.8/−3.2 kV; sheath gas flow rate at 40 arbitrary units (arb); auxiliary gas flow rate at 10 arb; auxiliary gas heater temperature at 350°C; and capillary temperature at 320°C. The full scan range was set from 70 to 1050 m/z with a resolution of 70,000, and the automatic gain control (AGC) target for mass spectrometry acquisitions was set to 3e6 with a maximum ion injection time of 100 ms. The top three precursors were selected for subsequent MS/MS fragmentation with a maximum ion injection time of 50 ms and a resolution of 30,000, while the AGC was set to 1e5. The stepped normalized collision energy was set to 20, 40, and 60 eV.

Network pharmacology analysis

Collection of bioactive compounds, target prediction, and disease-related genes

Nine bioactive compounds from ginger were selected based on an LC-MS/MS metabolite identification analysis for the ethanol extract of ginger rhizome. The chemical structures and simplified molecular-input line-entry system (SMILES) notations were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [23]. The SMILES notations were subsequently submitted to the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) to predict potential protein targets [24]. Predicted targets with probability scores greater than 0 were retained for further analysis, and the output included target proteins with their corresponding UniProt IDs and common names. Concurrently, breast cancer-related target genes were collected from the GeneCards database (https://www.genecards.org/) using "estrogen receptor positive breast cancer" as the search term [25]. Given that ER+ breast cancer represents approximately 70–80% of all breast cancer cases and is characterized by distinct molecular pathways amenable to targeted therapeutic interventions, focusing on this subtype allows for more refined target identification and reduces the dataset size for subsequent network analysis [7,26]. Gene symbols associated with ER-positive breast cancer were systematically extracted and compiled for integration with compound targets.

Identification of common targets

To identify overlapping targets between ginger compounds and breast cancer, a Venn diagram analysis was performed using the web-based tool available at http://bioinformatics.psb.ugent.be/webtools/Venn/. The common gene names from SwissTargetPrediction and gene symbols from GeneCards were uploaded to generate the intersection of compound-disease targets. These common targets represent potential therapeutic targets of ginger against breast cancer.

Gene ontology and pathway enrichment analysis

The intersecting targets were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using ShinyGO v0.77 (http://bioinformatics.sdstate.edu/go/) [27]. The enrichment analyses were performed with Homo sapiens as the reference species, and a false discovery rate (FDR) of < 0.05 was set as the significance threshold. Results were visualized as charts to illustrate the biological processes, molecular functions, cellular components, and signaling pathways involved in the anti-breast cancer effects of ginger.

Protein-protein interaction network construction

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 12.0 (https://string-db.org/) was utilized to construct a protein-protein interaction (PPI) network of the common targets [28]. The target gene symbols were imported into STRING with *Homo sapiens* selected as the organism, and a minimum interaction score of 0.4 (medium confidence)

was applied. The resulting PPI network was exported in tab-separated values (TSV) format for visualization and further analysis.

Network visualization and hub gene identification

The TSV file obtained from STRING was imported into Cytoscape software version 3.10.0 for network visualization and topological analysis [29]. The CytoHubba plugin was employed to identify hub genes based on network topology using multiple algorithms including Degree, Betweenness Centrality, and Closeness Centrality [30]. The top 10 hub genes with the highest connectivity scores were identified as key targets mediating the anti-breast cancer activity of ginger compounds. Network parameters such as node degree, betweenness centrality, and closeness centrality were calculated to evaluate the importance of each target in the network.

Molecular docking simulation

Blind molecular docking analyses were conducted using CB-Dock2, an advanced successor to the original CB-Dock web server [31]. This platform integrates curvature-based cavity recognition through the CurPocket algorithm, protein–ligand docking powered by the AutoDock Vina engine, and template-guided homologous structural alignment. CB-Dock2 provides a fully automated computational pipeline that encompasses unbiased detection of potential binding pockets, generation of three-dimensional grid parameters, adaptive optimization of docking search spaces based on ligand geometry, and systematic execution of docking simulations. The integration of these processes enhances computational efficiency while improving the robustness and accuracy of protein–ligand interaction predictions.

The selection of high-quality macromolecular structures is a critical factor influencing docking reliability and binding affinity estimation accuracy [32]. Three-dimensional crystal structures of hub target proteins were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB; https://rcsb.org; accessed January 6, 2026) [33] following strict inclusion criteria. These criteria comprised: (1) high crystallographic resolution (preferably < 2.5 Å) to ensure accurate atomic coordinates and minimize structural uncertainty [34]; (2) presence of co-crystallized ligands to facilitate reliable binding site identification and validation of the binding pocket conformation [35]; (3) completeness of the protein structure, particularly in the binding site region, to avoid artifacts from missing residues or loops; and (4) validation in recent cancer research, particularly breast cancer studies, to ensure relevance to the disease model under investigation [36,37].

Based on these parameters and supporting literature, the following protein structures were selected for the hub targets: AKT1 (PDB ID: 1UNQ) [38], GAPDH (PDB ID: 1U8F) [39], SRC (PDB ID: 1Y57) [40], STAT3 (PDB ID: 6NJS) [41], EGFR (PDB ID: 1M17) [42,43], ESR1 (PDBID: 3ERT) [44], PPARG (PDB ID: 4Y29) [45], MAPK3 (PDB ID: 4QTB) [46], MMP9 (PDB ID: 6ESM) [47], and TLR4 (PDB ID:4G8A) [48]. These entries were chosen due to their proven utility in cancer drug discovery and their demonstrated capacity to discriminate between active and inactive ligands, which is an essential prerequisite for reliable virtual screening performance [49]. Protein preprocessing, including the removal of crystallographic water molecules and non-protein heteroatoms, was automatically performed by the CB-Dock2 server. Subsequent docking efforts focused on hub proteins exhibiting high topological importance and substantial functional involvement in cancer-associated signaling pathways, as identified through network pharmacology analyses.

To establish comparative benchmarks for evaluating the binding performance of *Z. officinale*–derived compounds, molecular docking analyses were also performed using FDA-approved anticancer agents as reference standards. These included tamoxifen (PubChem CID: 2733526), a selective estrogen receptor modulator widely used as first-line endocrine therapy for estrogen receptor–positive breast cancer [4]; doxorubicin (PubChem CID: 31703), an anthracycline chemotherapeutic agent central to breast cancer treatment across multiple molecular subtypes [6]; and 5-fluorouracil (PubChem CID: 3385), a fluoropyrimidine antimetabolite frequently incorporated into combination chemotherapy regimens [5]. Collectively, these reference compounds represent distinct therapeutic mechanisms, including estrogen receptor antagonism, DNA intercalation with topoisomerase II inhibition, and

thymidylate synthase inhibition. Incorporating both targeted and cytotoxic agent enables a comprehensive evaluation of ginger phytochemicals across diverse anticancer modalities, facilitating assessment of their potential as multifunctional natural therapeutics in breast cancer treatment. Structural data for all reference ligands were retrieved from the PubChem database and prepared using the same molecular processing protocols applied to the ginger-derived compounds.

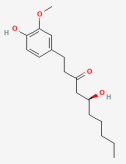
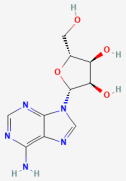
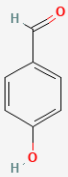
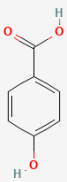
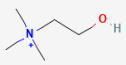
Ligand structures were obtained from the PubChem repository in Structure Data File (SDF) format (https://pubchem.ncbi.nlm.nih.gov; accessed January 6, 2026). Binding affinities between ligands and target proteins were calculated using the AutoDock Vina scoring function, with more negative binding energy values indicating stronger predicted interactions [31]. The docked poses exhibiting the lowest binding free energies were further analyzed using BIOVIA Discovery Studio Visualizer to systematically characterize key intermolecular interactions, including hydrogen bonds, hydrophobic interactions, and other non-covalent forces contributing to ligand–receptor stabilization.

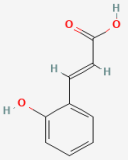
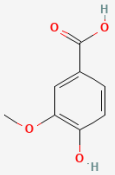
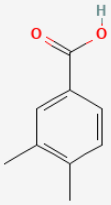
Results

Compounds in ginger extract

The observed compounds were automatically identified through the mzCloud MS/MS Library, and 10 molecules were found to have a match of >99%. Molecular formula, retention time, and calculated molecular weight for compounds identified from non-targeted metabolomics profiling via LC-MS/MS are summarized in **Table 1**.

Table 1. Compounds in *Zingiber officinale* identified by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS)

Observed compounds	Molecular formula	RT (min)	Calculated MW (m/z)	PubChem ID	Annotated delta mass (ppm)	Molecular structure
6-gingerol	$C_{17}H_{26}O_4$	8.736	294.18	442793	-0.7	
Adenosine	$C_{10}H_{13}N_5O_4$	0.863	267.09	60961	-1.58	
4-Hydroxy-benzaldehyde	$C_7H_6O_2$	2.929	122.03	126	-1.38	
4-Hydroxy-benzoic acid	$C_7H_6O_3$	5.009	138.03	135	-1.79	
Choline	$C_5H_{13}NO$	0.644	103.09	305	1.51	

Observed compounds	Molecular formula	RT (min)	Calculated MW (m/z)	PubChem ID	Annotated delta mass (ppm)	Molecular structure
2-Hydroxy-cinnamic acid	$C_9H_8O_3$	3.461	164.04	637540	-1.98	
Vanillic acid	C8H8O4	2.503	168.04	8468	-1.78	
3,4-Dimethyl-benzoic acid	$C_9H_{10}O_2$	17.758	150.06	12073	-0.66	
16-Hydroxy-hexadecanoic acid	C16H32O3	14.209	272.23	10466	-1.21	

Network pharmacology analysis

Nine bioactive compounds were identified from ginger ethanol extract through LC-MS/MS analysis. These compounds included 6-gingerol, adenosine, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, choline, 2-hydroxycinnamic acid, vanillic acid, 3,4-dimethylbenzoic acid, and 16-hydroxyhexadecanoic acid. Drug-likeness evaluation revealed that all nine compounds satisfied Lipinski's Rule of Five, indicating favorable oral bioavailability profiles. However, two compounds (6-gingerol and 16-hydroxyhexadecanoic acid) were rejected by the Pfizer Rule, and one compound (16-hydroxyhexadecanoic acid) failed to meet GSK Rule criteria, suggesting potential limitations in their development as conventional oral drugs. Toxicity prediction analysis classified the compounds into toxicity classes ranging from 2 to 5, with predicted LD_{50} values between 8 mg/kg (adenosine) and 2,850 mg/kg (2-hydroxycinnamic acid), demonstrating relatively low acute toxicity for most compounds.

Target prediction via SwissTargetPrediction identified a total of 445 potential protein targets for the nine ginger compounds with probability scores >0. Simultaneously, a search of the GeneCards database using "estrogen receptor positive breast cancer" as the keyword yielded 10,003 disease-related genes. Venn diagram analysis revealed 266 overlapping targets between ginger compounds and ER-positive breast cancer (**Figure 1**), suggesting these common targets as potential therapeutic intervention points for ginger's anti-breast cancer activity. The analysis reveals three distinct domains of protein-target interaction. The left circle represents 335 total targets associated with ginger (69 unique targets plus 266 shared targets). In comparison, the right circle encompasses 10,003 total protein targets related to breast cancer (9,737 unique targets plus 266 shared targets). Most significantly, the overlapping region identifies 266 genes and proteins that represent the intersection targets associated with both breast cancer and ginger, suggesting these shared targets may be the key molecular mediators through which ginger exerts its therapeutic effects against breast cancer. The 69 ginger-specific targets indicate unique bioactive mechanisms not directly related to breast cancer pathways. In comparison, the 9,737 breast cancer-specific targets represent disease-associated proteins that are not modulated by ginger compounds.

To elucidate the gene networks modulated by ginger, KEGG pathway enrichment analysis was performed. The analysis revealed the top 20 enriched pathways (**Figure 2**), including EGFR tyrosine kinase inhibitor resistance, non-small cell lung cancer, HIF-1 signalling pathway, PD-L1

expression and PD-1 checkpoint pathway in cancer, endocrine resistance, prostate cancer, AGE-RAGE signaling pathway in diabetic complications, estrogen signaling pathways, lipid and atherosclerosis, CAMP signaling pathway, human cytomegalovirus infection, hormone signaling, neuroactive ligand-receptor interaction, calcium signaling pathway, pathways in cancer, PI3K-Akt signalling pathway, and metabolic pathways. Among the enriched pathways, the EGFR tyrosine kinase inhibitor resistance pathway warrants particular attention (**Figure 3**).

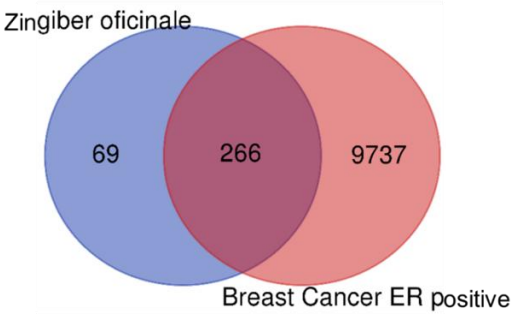


Figure 1. Venn diagram showing the shared targets of *Zingiber officinale* and genes associated with breast cancer.

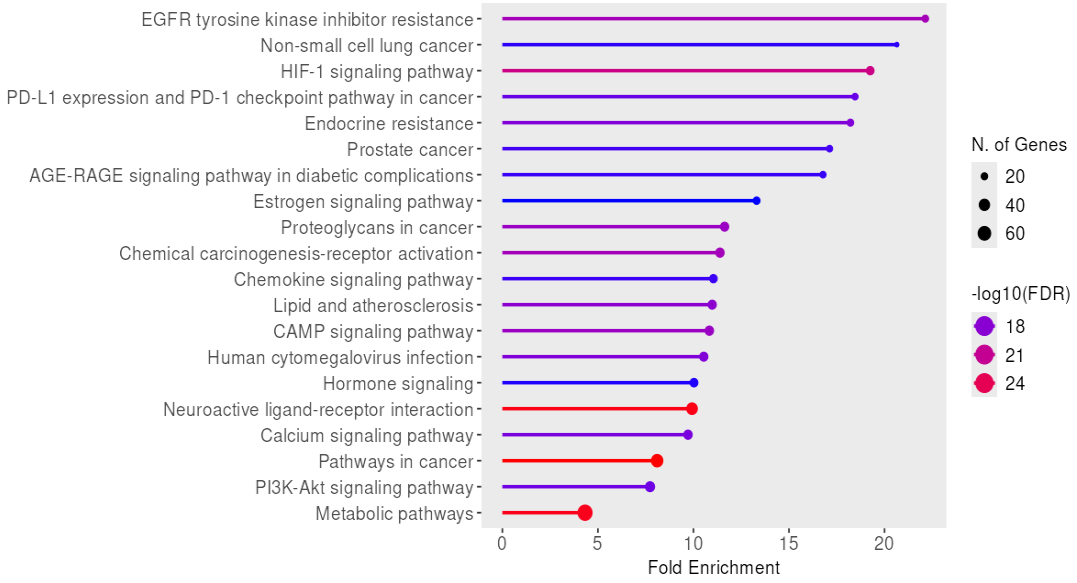


Figure 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the most enriched pathways.

The complex molecular mechanisms underlying resistance to EGFR-targeted therapies are illustrated in **Figure 3**, which often share common signaling nodes with ER-positive breast cancer progression. The pathway shows multiple hit targets (indicated in red) that are modulated by ginger compounds, including key proteins in the MAPK signaling cascade (MAPK1/ERK, MAPK3/ERK), PI3K-Akt signaling (PIK3CA, AKT), STAT signaling (STAT3, STAT5), and apoptosis regulation (Bcl-2, Bcl-XL). The presence of EGFR, IGF1R, FGFR, VEGFR, and PDGFR among the affected targets suggests that ginger compounds may simultaneously inhibit multiple receptor tyrosine kinases, thereby preventing compensatory signaling that often leads to therapeutic resistance. Furthermore, the pathway shows that ginger compounds target both upstream receptors and downstream effectors, including transcription factors and cell cycle regulators, which may result in synergistic anti-cancer effects. The modulation of mTOR signaling components (mTORC1, S6) indicates potential effects on protein synthesis and cell growth. In contrast, the targeting of apoptosis-related proteins suggests that ginger compounds may restore programmed cell death in cancer cells that have acquired resistance mechanisms.

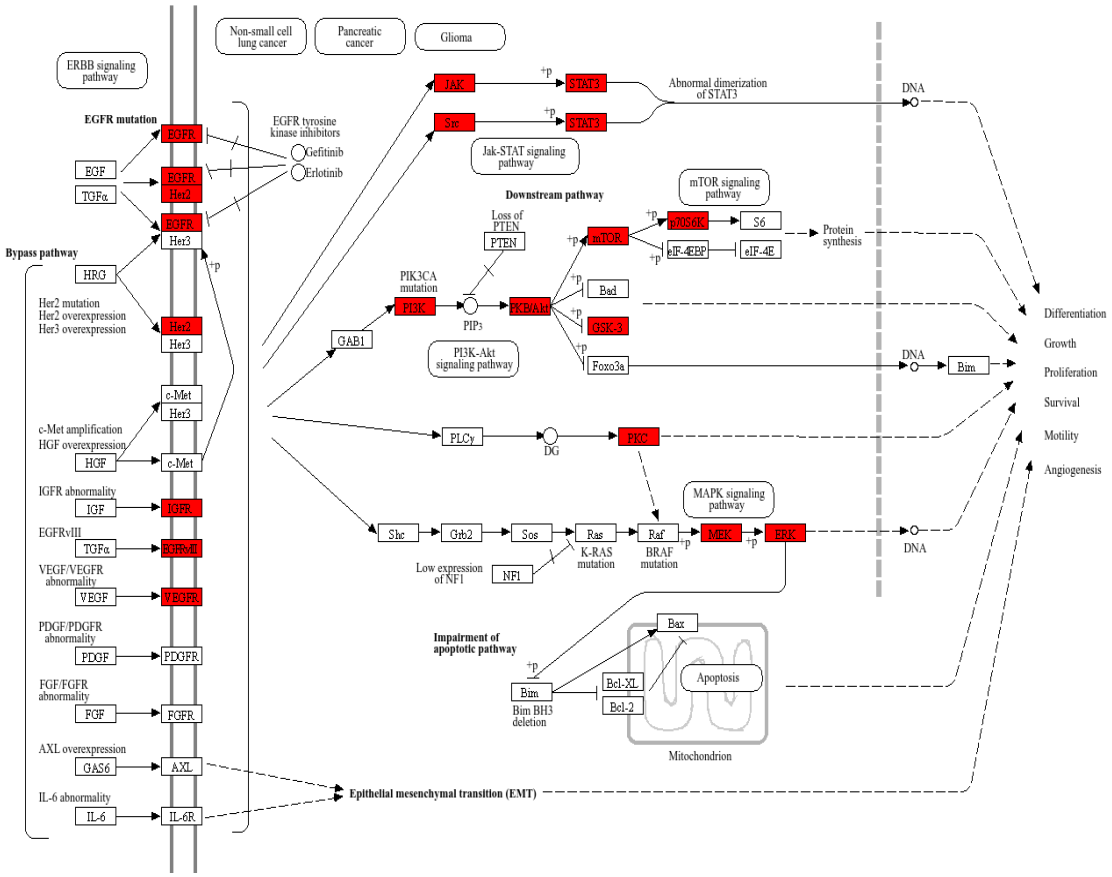


Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway diagram analysis of *Zingiber officinale* target genes (highlighted in red) in breast cancer.

To further explore the interactions among the 266 common targets, a protein-protein interaction (PPI) network was constructed using the STRING database and visualized in Cytoscape. Hub gene analysis was performed using the CytoHubba plugin in Cytoscape based on degree centrality. The top 10 hub genes identified were *AKT1, GAPDH, SRC, STAT3, EGFR, ESR1, PPARG, MAPK3, MMP9*, and *TLR4* (**Figure 4**). These hub genes exhibited the highest connectivity scores in the network, indicating their central roles in mediating the anti-breast cancer effect of ginger compounds.

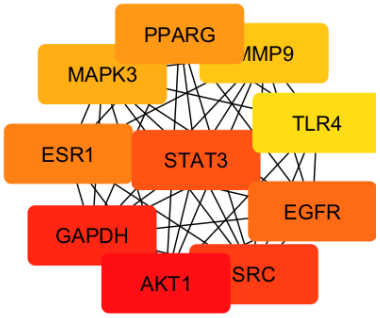


Figure 4. Top 10 hub genes in protein-protein interaction network ranked by degree centrality. Color coding reflects connectivity ranking: red (highest degree: *AKT1, GAPDH, SRC*), orange (intermediate degree), and yellow (lowest degree). Lines represent validated interactions between *Zingiber officinale* targets and breast cancer-related proteins.

Molecular docking simulation

To validate the predicted therapeutic target from network pharmacology analysis, molecular docking was performed to assess the binding affinities of nine ginger compounds and three FDA-approved reference drugs against the ten hub proteins. The molecular docking results of all compound-target interactions are presented in **Table 2**. The selected protein targets: AKT1,

GAPDH, SRC, STAT3, EGFR, ESR1, PPARG, MAPK3, MMP9, and TLR4, represent critical pathways involved in breast cancer metabolism, proliferation, invasion, hormone signaling, and immune modulation. Clinically approved therapeutic agents including tamoxifen, doxorubicin, and 5-fluorouracil served as control standards in the docking protocol.

Among the ginger compounds, two emerged as the most promising candidates based on their strong binding affinities: adenosine and 6-gingerol (**Table 2**). Adenosine demonstrated the strongest overall binding, with exceptional affinity to GAPDH (–9.0 kcal/mol)—approaching that of tamoxifen's binding to the same target (–8.9 kcal/mol), followed by robust binding to MAPK3 (–8.0 kcal/mol), MMP9 (–8.0 kcal/mol), SRC (–7.5 kcal/mol), ESR1 (–7.5 kcal/mol), and TLR4 (–7.3 kcal/mol) (**Table 2**).

6-Gingerol, the major bioactive constituent of ginger, exhibited strong binding particularly to MMP9 (–7.9 kcal/mol), ESR1 (–7.6 kcal/mol), MAPK3 (–7.6 kcal/mol), and GAPDH (–7.2 kcal/mol) (**Table 2**). Target-specific analysis revealed that GAPDH was the most receptive to ginger compounds, with adenosine and 6-gingerol all showing binding energies below –7.0 kcal/mol, suggesting metabolic disruption as a primary anti-cancer mechanism. MMP9 similarly demonstrated high susceptibility, with four compounds exhibiting strong binding (<–7.0 kcal/mol), indicating that MMP9 inhibition and anti-metastatic effects constitute a key therapeutic mechanism of ginger. MAPK3 and ESR1 also showed favorable binding, validating these proteins as critical targets identified through network pharmacology.

Detailed interaction analysis revealed the molecular basis for these strong binding affinities (**Table 3**, **Figure 5**). The adenosine-GAPDH complex showed multiple hydrogen bonds with key residues including THR65, ARG15, GLU17, THR87 (chain O), and ASN239 (chain P), along with extensive hydrophobic interactions involving THR237, VAL240, ASN287, THR52, SER51, and ALA238.

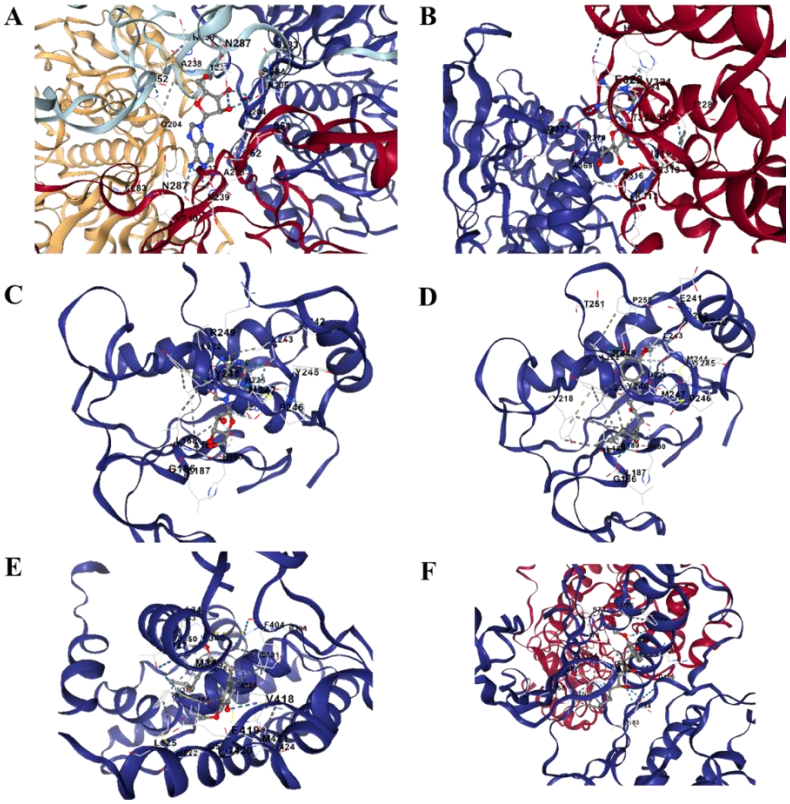


Figure 5. The best ten docked amino acid residue interactions: (A) Adenosine with GAPDH; (B) Adenosine with MAPK3; (C) Adenosine with MMP9; (D) 6-gingerol with MMP9; (E) 6-gingerol with ESR1; (F) 6-gingerol with MAPK3.

Table 2. Molecular docking analysis of *Zingiber officinale* bioactive compounds with breast cancer target proteins using AutoDock Vina

Name	AKT1 (1UNQ)	GAPDH (1U8F)	SRC (1Y57)	STAT3 (6NJS)	EGFR (1M17)	ESR1 (3ERT)	PPARG (4Y29)	MAPK3 (4QTB)	MMP9 (6ESM)	TLR4 (4G8A)
6-Gingerol	−5.4	−7.2	−6.6	−5.9	−6.0	−7.6	−6.7	−7.6	−7.9	−6.9
Adenosine	−5.9	−9.0	−7.5	−6.4	−6.9	−7.5	−6.6	−8.0	−8.0	−7.3
4-Hydroxy-benzaldehyde	−4.6	−5.6	−4.9	−4.8	−5.5	−5.3	−5.1	−5.4	−6.0	−5.5
4-Hydroxybenzoic acid	−5.1	−6.0	−5.4	−5.0	−6.1	−5.7	−5.4	−5.8	−6.4	−5.9
Choline	−3.2	−3.7	−3.5	−3.2	−3.5	−3.7	−3.5	−3.7	−3.5	−3.7
2-Hydroxycinnamic acid	−5.4	−6.5	−6.5	−5.6	−5.8	−6.5	−5.8	−6.8	−7.8	−6.3
Vanillic acid	−5.1	−6.1	−5.4	−5.2	−6.1	−5.9	−5.6	−6.0	−6.7	−6.2
3,4-Dimethylbenzoic acid	−5.2	−6.5	−6.0	−5.5	−5.7	−6.2	−5.7	−6.1	−7.3	−6.8
16-Hydroxyhexadecanoic acid	−4.3	−5.8	−5.3	−5.1	−5.2	−5.9	−5.6	−6.3	−6.3	−6.2
Tamoxifen	−5.5	−8.9	−7.8	−6.6	−7.4	−9.3	−8.2	−8.5	−7.3	−8.2
Doxorubicin	−7.2	−12.8	−8.0	−7.7	−8.8	−8.0	−7.5	−10.0	−7.6	−9.7
5-fluorouracil	−4.4	−5.5	−4.8	−4.7	−4.9	−5.3	−5.3	−5.7	−5.9	−5.6

Values are binding affinities in kcal/mol; more negative indicates stronger binding

Table 3. Amino acid residue interactions between ginger bioactive compounds and key breast cancer hub proteins identified by molecular docking

Name	AKT1 (1UNQ)	GAPDH (1U8F)	SRC (1Y57)	STAT3 (6NJS)	EGFR (1M17)	ESR1 (3ERT)	PPARG (4Y29)	MAPK3 (4QTB)	MMP9 (6ESM)	TLR4 (4G8A)
Adenosine	Chain A: H: ARG67, PRO68, ILE74, THR72, GLY16, LYS20 HB: THR65, ARG15, GLU17, THR87	Chain O: H: THR65, ARG15, GLU17, THR87 HB: THR237, VAL240, ASN287, THR52, SER51, ALA238 Chain P: H: ASN239 HB: GLN204 Chain R: H: ASN205 HB: GLN204 Chain Q: H: THR237, ASN239, THR52, ALA238 HB:	Chain A: H: PHE405, ILE336, THR338, LYS295, ILE294, VAL281, GLY344, LEU273, SER345, TYR340, LEU393, MET341, VAL323, ALA293, ALA403, MET314 HB: ASP404, GLU310	Chain A: H: GLU435, LEU430, ILE431, ARG379, LYS551 HB: VAL490, LEU436, PHE493, GLU434, ASN491, SER381, THR494	Chain A: H: PHE832, THR830, MET742, CYS751, VAL702, GLY772, LEU694, LEU768, PRO770, GLN767, LEU820, ALA719, ASP831, HB: THR766, GLU738, LYS721, MET769	Chain A: H: LEU349, LEU387, ARG394, LEU391, LEU428, MET421, LEU384, GLY521, ILE424, MET388, HIS524, MET343, LEU525, PHE404, ALA350 HB: GLU353, LEU346	Chain A: H: ARG288, ILE341, CYS285, GLY284, MET348, ILE281, VAL277, LEU255, GLU259, LYS261, ILE262 HB: SER342, ARG280	Chain A: H: THR320, VAL321, HIS158, LYS224, ILE228, ARG318, ASN316, LYS317 HB: GLU322, PRO315, SER225 Chain B: H: ARG370 HB: ALA369	Chain A: H: ALA189, LEU188, GLN227, HIS226, ALA225, LEU243, ARG249, TYR245, MET247, PRO246, GLY186, LEU187 HB: TYR248, LEU222, VAL223	Chain A: H: SER211, ASP209, ASP181, HIS179, LEU208, LYS230, ARG234, PHE263 HB: THR232, LEU180, SER207 Chain C: H: SER98, ARG106, ASP100 HB: ASP99

Name	AKT1 (1UNQ)	GAPDH (1U8F)	SRC (1Y57)	STAT3 (6NJS)	EGFR (1M17)	ESR1 (3ERT)	PPARG (4Y29)	MAPK3 (4QTB)	MMP9 (6ESM)	TLR4 (4G8A)
		SER284, ASN287								
6-Gingerol	Chain A: H: LYS20, ILE74, GLU17, ARG86, VAL83, ILE84, GLU85, PRO68, ARG67 HB: ARG15, GLY16, THR87	Chain O: H: GLN204 Chain P: H: SER51, SER284, THR52, VAL240, ASN287, THR237 HB: ALA238, ASN239 Chain Q: H: GLN204 Chain R: H: THR52, ALA238, ASN239 HB: SER284, ASN287	Chain A: H: PHE150, TYR149, TYR90, SER248, LEU89, GLU146, GLY151 HB: THR247, GLN144, GLU147	Chain A: H: HIS457, ASP371, LYS383, LYS488, LYS370, LEU438, VAL490, HIS437 HB: GLU455, THR440, ASP369, LEU436	Chain A: H: GLY772, LEU768, ALA719, LEU820, THR766, LEU764, THR830, PHE832, GLU738, ASP831, MET742, LEU694, VAL702, GLY695 HB: MET769, LYS721	Chain A: H: ILE424, MET343, PHE404, MET388, MET421, LEU391, LEU346, LEU387, THR347, ALA350, TRP383, LEU349, LEU525, LEU384, GLY521 HB: ARG394, GLU353	Chain A: H: ARG288, CYS285, LEU465, LEU453, GLN286, LEU469, PHE282, HIS449, MET329, ILE326, LEU330, LEU333, ALA292 HB: SER289, HIS323, TYR473, TYR327	Chain B: H: CYS183, GLU88, PRO75, ILE73, THR85, ALA52, ARG84, LYS71, VAL56 HB: ASP184, GLY186, SER74, TYR53, TYR81	Chain A: H: ALA189, LEU187, LEU188, PRO246, VAL223, TYR245, HB: ALA242, PRO255, THR251, LEU222, HIS226, TYR248, LEU243, GLY186, TYR218,GLN227, ARG249, MET247	Chain B: H: PHE440, PHE463, ASN417, LEU444 HB: SER441 Chain C: H: ILE124, LEU87, ILE80, CYS133, ILE153, ILE52, LEU54, TYR131, VAL82, PHE126, SER127 HB: LYS125

H: hydrogen; HB: hydrophobic

The 6-gingerol-MMP9 complex demonstrated hydrogen bonding with ALA189, LEU187, LEU188, PRO246, VAL223, TYR245, and extensive hydrophobic contacts including ALA242, PRO255, THR251, LEU222, HIS226, TYR248, LEU243, GLY186, and TYR218, with additional interactions via GLN227, ARG249, and MET247 (**Table 3**, **Figure 5**). These interactions position 6-gingerol within the MMP9 catalytic site, potentially interfering with substrate binding and enzymatic activity.

When benchmarked against FDA-approved drugs, ginger compounds showed competitive binding affinities in several instances (**Table 2**). Adenosine binding to GAPDH (–9.0 kcal/mol) was comparable to tamoxifen (–8.9 kcal/mol) and approached the affinity range of doxorubicin. Similarly, 6-gingerol binding to MMP9 (–7.9 kcal/mol) exceeded that of tamoxifen (–7.3 kcal/mol), suggesting potential anti-metastatic activity through MMP9 inhibition. Although ginger compounds generally had weaker binding than doxorubicin, several constituents showed affinities within the therapeutic range (–6.0 to –9.0 kcal/mol) commonly associated with bioactive compounds. These findings support the potential of ginger-derived compounds, particularly adenosine and 6-gingerol, as lead structures for breast cancer drug development or complementary natural therapeutics that may enhance conventional treatments through synergistic multi-target modulation.

Discussion

The present study combined LC–MS/MS profiling, network pharmacology, and molecular docking approaches to explore the potential anti-breast cancer mechanisms of *Z. officinale* against ER-positive breast cancer. The findings suggest that ginger exerts multi-target regulatory effects through simultaneous modulation of oncogenic signaling, metabolic adaptation, inflammatory pathways, and extracellular matrix remodeling. This systems-level activity is particularly relevant in ER-positive breast cancer, where therapeutic resistance often arises from compensatory activation of interconnected molecular pathways rather than a single oncogenic driver [20,21]. LC–MS/MS analysis identified several bioactive constituents in ginger extract, including 6-gingerol, adenosine, vanillic acid, hydroxycinnamic acid derivatives, and phenolic acids. Among these compounds, 6-gingerol has been extensively recognized as the principal pharmacologically active constituent of ginger with documented anti-inflammatory, antioxidant, and anti-cancer activities [22,23]. Previous studies demonstrated that 6-gingerol suppresses breast cancer cell proliferation through induction of apoptosis, inhibition of NF-κB signaling, and suppression of metastatic behavior [24]. The identification of adenosine as another major candidate is also noteworthy, as purinergic signaling has increasingly been implicated in cancer metabolism, immune regulation, and tumor progression [25].

The network pharmacology analysis revealed 266 overlapping targets between ginger-derived compounds and ER-positive breast cancer-associated genes, supporting the concept that phytochemicals exert therapeutic activity through polypharmacological mechanisms [26]. Unlike conventional targeted therapies that often focus on a single receptor or pathway, natural products frequently influence multiple signaling nodes simultaneously, thereby reducing the likelihood of adaptive resistance [27]. In the context of ER-positive breast cancer, resistance to endocrine therapy remains a major clinical challenge and commonly involves activation of PI3K/AKT, MAPK, EGFR, and inflammatory signaling pathways [28,29]. The identification of these pathways in the present analysis therefore strengthens the biological plausibility of ginger as a complementary therapeutic candidate. KEGG enrichment analysis further demonstrated significant involvement of pathways associated with EGFR tyrosine kinase inhibitor resistance, endocrine resistance, HIF-1 signaling, PI3K-Akt signaling, and PD-L1 checkpoint regulation. Crosstalk between ER signaling and growth factor receptor pathways such as EGFR and HER2 is known to contribute substantially to therapeutic escape in luminal breast cancer [30]. Activation of PI3K/AKT and MAPK signaling enhances cellular survival, proliferation, and resistance to apoptosis, ultimately reducing responsiveness to endocrine therapy [31]. The observation that ginger-associated targets were distributed across both upstream receptors and downstream signaling mediators suggests that ginger compounds may interfere with multiple compensatory survival pathways simultaneously.

The enrichment of HIF-1 signaling is particularly relevant because hypoxia-driven metabolic reprogramming plays a central role in breast cancer progression and therapeutic resistance [32]. HIF-1α activation promotes angiogenesis, glycolytic adaptation, epithelial–mesenchymal transition (EMT), and metastatic dissemination [33]. Ginger-derived phytochemicals can suppress HIF-1α accumulation and reduce VEGF-mediated angiogenesis under hypoxic conditions [34]. These effects may partially explain the predicted anti-cancer activity observed in the current network analysis. PPI analysis identified *AKT1, GAPDH, SRC, STAT3, EGFR, ESR1, PPARG, MAPK3, MMP9*, and *TLR4* as the major hub genes. These proteins collectively regulate critical biological processes including proliferation, inflammatory signaling, estrogen responsiveness, invasion, and metabolic adaptation [35]. AKT1 is a master regulator of cell survival and metabolism and is frequently hyperactivated in ER-positive breast cancer through PI3K pathway alterations [36]. Similarly, SRC and STAT3 signaling contribute to endocrine resistance, tumor invasiveness, and cancer stemness [37]. Persistent activation of STAT3 has been associated with poor prognosis and increased metastatic potential in breast cancer patients [38].

Among the identified targets, GAPDH emerged as one of the most interesting proteins due to its exceptionally strong interaction with adenosine. Although GAPDH is classically recognized as a glycolytic enzyme, increasing evidence indicates that it also participates in transcriptional regulation, apoptosis, DNA repair, and tumor metabolic adaptation [39]. Cancer cells frequently exhibit elevated glycolytic activity even under normoxic conditions, a phenomenon known as the Warburg effect [40]. The strong docking affinity observed between adenosine and GAPDH suggests that ginger compounds may disrupt metabolic plasticity in breast cancer cells, thereby limiting cellular energy production and biosynthetic capacity. MMP9 also demonstrated strong interactions with 6-gingerol and several other ginger-derived compounds. MMP9 is critically involved in extracellular matrix degradation, tumor invasion, angiogenesis, and metastatic dissemination [41]. Elevated MMP9 expression has been correlated with poor prognosis and increased metastatic burden in breast cancer [42]. A previous experimental study demonstrated that 6-gingerol suppresses migration and invasion of breast cancer cells through inhibition of MMP expression and EMT-associated signaling [43]. The current docking findings are therefore consistent with earlier biological observations and support the potential anti-metastatic activity of ginger constituents.

The interaction between 6-gingerol and ESR1 is also biologically meaningful because ER-positive breast cancer is fundamentally driven by estrogen receptor signaling. Tamoxifen resistance frequently develops through receptor mutation, altered co-regulator recruitment, or bypass activation of growth factor pathways [44]. Natural compounds capable of modulating ESR1 activity while simultaneously suppressing compensatory signaling pathways may therefore provide therapeutic benefit. Previous investigations reported that ginger extracts can influence estrogen-responsive signaling and induce apoptotic responses in hormone-dependent breast cancer models [45]. The observed interactions with EGFR, MAPK3, and TLR4 further suggest that ginger compounds may modulate inflammatory and proliferative signaling simultaneously. EGFR signaling contributes not only to tumor proliferation but also to resistance against endocrine therapies [46]. MAPK activation is similarly associated with uncontrolled proliferation and survival signaling in breast cancer [47]. Meanwhile, TLR4 activation within the tumor microenvironment promotes inflammatory cytokine production, immune evasion, and metastatic progression [48]. The ability of ginger compounds to interact with these proteins supports the concept that ginger exerts integrated anti-inflammatory and anti-oncogenic activities.

An important observation from this study is that several ginger compounds demonstrated docking affinities approaching those of established anti-cancer drugs. Adenosine exhibited strong interaction with GAPDH comparable to tamoxifen, while 6-gingerol demonstrated stronger binding to MMP9 than tamoxifen in the current docking model. Although docking affinity alone cannot predict therapeutic efficacy, these findings suggest that ginger-derived compounds possess structurally favorable interactions with clinically relevant molecular targets [49]. Similar observations have been reported for other plant-derived polyphenols and flavonoids that exhibit moderate-to-strong affinity toward oncogenic proteins while simultaneously maintaining lower

systemic toxicity [50]. The multi-target profile observed in this study may also be advantageous in overcoming therapeutic resistance. Cancer cells possess remarkable adaptive capacity and frequently activate alternative signaling pathways in response to selective pharmacological pressure [51]. Agents capable of simultaneously targeting metabolic pathways, inflammatory mediators, receptor signaling, and extracellular remodeling may therefore produce broader anti-tumor effects than highly selective single-target drugs [52]. This concept aligns with growing interest in network pharmacology-based therapeutic strategies for complex diseases such as cancer. In addition to direct anti-cancer activity, ginger-derived compounds may also provide supportive benefits through modulation of oxidative stress and inflammatory burden. Chronic inflammation contributes substantially to breast cancer progression, immune dysregulation, and metastatic development [53]. Ginger has long been recognized for its anti-inflammatory activity through suppression of NF-κB, COX-2, TNF-α, and IL-6 signaling [54]. These mechanisms may contribute indirectly to suppression of tumor-promoting inflammation within the tumor microenvironment.

Despite these promising findings, several limitations should be acknowledged. First, the present study relied predominantly on computational prediction models, which cannot fully replicate the complexity of biological systems. Molecular docking evaluates theoretical ligand–protein affinity but does not account for pharmacokinetics, cellular uptake, metabolism, or dynamic intracellular interactions [55]. Second, the bioavailability of several ginger-derived compounds may be limited in vivo due to rapid metabolism and poor systemic stability. Third, the predicted therapeutic mechanisms require validation through in vitro and in vivo experimental studies using ER-positive breast cancer models. Future studies should therefore investigate the effects of ginger extract and isolated compounds on breast cancer cell viability, apoptosis, migration, endocrine resistance, and metabolic reprogramming. Functional assays evaluating PI3K/AKT, MAPK, STAT3, and HIF-1α signaling would further clarify the mechanistic relevance of the predicted pathways. In vivo studies examining tumor growth inhibition and metastatic suppression are also necessary to confirm translational potential. Moreover, combination studies with tamoxifen or aromatase inhibitors may help determine whether ginger-derived compounds can enhance endocrine responsiveness or reduce resistance development.

Conclusion

This study elucidates the multi-target anti-breast cancer mechanisms of *Z. officinale* through an integrated network pharmacology and molecular docking approach. Network analysis identified 266 overlapping targets and ten key hub proteins (AKT1, GAPDH, SRC, STAT3, EGFR, ESR1, PPARG, MAPK3, MMP9, and TLR4), highlighting ginger's capacity to modulate critical pathways involved in cancer metabolism, proliferation, invasion, hormone signaling, and immune regulation. Molecular docking validated these targets and identified adenosine and 6-gingerol as the most promising bioactive compounds, with particularly strong interactions observed for adenosine–GAPDH and 6-gingerol–MMP9, suggesting metabolic and anti-metastatic mechanisms. The convergence of multiple ginger constituents on common targets such as GAPDH and MMP9 supports a synergistic, systems-level mode of action consistent with traditional herbal use. Overall, these findings provide molecular-level evidence that ginger exerts therapeutic effects through coordinated multi-component, multi-target interactions, supporting its potential as a complementary therapeutic or chemopreventive agent in estrogen receptor-positive breast cancer and offering a rational basis for future experimental and clinical validation.

Ethics approval

Not required.

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None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

All data have been presented as part of the article.

Declaration of artificial intelligence use

The authors confirm that no artificial intelligence (AI) tools or methodologies were used at any stage of this study, including data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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