Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.15 (2): 28-44; June 2026 *ISSN: 2971-737X (Print); ISSN: 2971-7388.*
Available at www.nijophasr.net *https://doi.org/10.60787/nijophasr-v15-i2-655*

Deciphering the Multi-Target Anti-Breast Cancer Mechanisms of *Vernonia amygdalina* (Bitter Leaf) through Network Pharmacology and Molecular Docking

[1*]Toheeb A. Sodiq, [2]Abdulrazaq Sanusi, [3]Adeyemi S. Febilola [4]Mmesoma E. Ernest-Eze and [5]Munachi I. Okeke

1. Department of Pharmacology, Sa'adu Zungur University, Gadau, Bauchi State, Nigeria
2. Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria
3. Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.
4. Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria
5. College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria

Article info: Volume 15, Issue 2, June 2026; Received: May 15, 2026; Reviewed: May 23, 2026, Accepted: June 6, 2026; Published: June 15, 2026; *DOI: 10.60787/nijophasr-v14-i2-651*

ABSTRACT

***Background*:** Breast cancer remains the leading cause of cancer-related mortality among women globally, necessitating novel multi-target therapeutic strategies. This study employed an integrated network pharmacology and molecular docking approach to elucidate the anti-breast cancer mechanisms of *Vernonia amygdalina* (bitter leaf).

Methods: Five bioactive phytochemicals, 11,13-dihydrovernodalin, hydroxyvernolide, vernodalin, vernolide, and vernomygdin were identified following ADMET and drug-likeness screening.

***Results*:** Venn diagram analysis revealed 21 overlapping targets between *V. amygdalina* phytochemicals and breast cancer-associated genes. Protein–protein interaction network analysis identified Aurora Kinase A (AURKA) and Thymidylate Synthase (TYMS) as principal hub proteins (PPI enrichment $p = 9.18 \times 10^{-8}$). Gene Ontology and KEGG pathway enrichment analyses implicated kinase activity, mitotic spindle regulation, and the PI3K-Akt, MAPK, and Ras signaling cascades as key modulated functions and pathways. Molecular docking confirmed strong binding affinities, with hydroxyvernolide and vernomygdin exhibiting the highest affinity toward TYMS (−8.6 kcal/mol) and 11,13-dihydrovernodalin toward AURKA (−8.0 kcal/mol).

***Conclusion*:** These findings demonstrate that *V. amygdalina* exerts anti-breast cancer effects through a multi-component, multi-target mechanism, providing a strong computational rationale for further experimental validation.

Keywords: Breast cancer, Molecular docking, Network pharmacology, Phytochemicals, Vernonia amygdalina;

1.0 INTRODUCTION

2.3 million women worldwide received a breast cancer diagnosis in 2022, which led to 670,000 fatalities. It can happen at any age after puberty and affects women worldwide, with incidence rates increasing with age [1]. The most common cause of cancer-related death in women is breast cancer. More than half of breast cancer diagnoses and two-thirds of breast cancer deaths in 2020 occurred in less developed regions of the world, despite the fact that the disease was previously thought to be primarily associated with industrialized nations [2,3]. Due to changes in the prevalence and distribution of important cancer risk factors, many of which are associated with socioeconomic development, as well as population ageing and expansion, the worldwide burden of cancer incidence and mortality is generally rising

*corresponding author: Email: toheebayodejisodiq@gmail.com; Phone: +2349161435193

quickly [4,5]. But new data also shows an alarming increase in early-onset breast cancer, especially in women under 50, who as of 2019 had the highest cancer-related DALYs in this age group worldwide [6]. Even if the overall incidence of breast cancer continues to rise with age, studies like Zhao et al. have shown an increasing trend in the prevalence of early-onset breast cancer, which may be related to lifestyle modifications, environmental exposures, and genetic predispositions [6]. The need for thorough predictions that take age-specific dynamics into account is highlighted by the dual trend of increased cases in both younger and older groups. Cancer, characterized by the unwanted proliferation of cells arising from an aggregation of various genetic anomalies, including dysregulation of genes and their regulators, has a deep-rooted history dating back approximately 1.98 million years. Evidence of tumors, specifically osteosarcoma, has been discovered in human fossils from South Africa, showcasing the ancient existence of this disease [7]. *Vernonia amygdalina*, variously known as bitter leaf (English), oriwo (Edo), ewuro (Yoruba), shikawa (Hausa), and olubu (Igbo), is a tropical shrub, 1-3m in height with petiole leaf of about 6mm in diameter, and elliptic in shape. The leaves are dark green coloured with a characteristic odour and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub- Saharan Africa. The leaves are eaten, after crushing and washing thoroughly to remove the bitterness. All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others. The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are used to prepare the very popular bitter leaf soup in Nigeria and are also reportedly consumed by goats in some parts of Nigeria. Antihelmitic and antimalarial properties as well as antitumorigenic properties, have also been reported for extracts from the plant [8]. The exact chemical mechanisms underlying bitter leaf's pharmacological actions are still unknown, despite the plant's long history of traditional use and proof of its effectiveness. Sometimes the complex interactions between bioactive chemicals in plant extracts and their effects on cellular signalling pathways are too complex for traditional pharmacological approaches to fully understand. However, using computational approaches and systems biology, network pharmacology has become a powerful paradigm for understanding the complex relationships between medicines, targets, and disorders inside biological networks [9, 10]. In order to systematically elucidate the multi-target anti-breast cancer mechanisms of *Vernonia amygdalina* using network pharmacology integrated with molecular docking validation, this approach systematically explores the molecular landscape underlying the therapeutic qualities of natural compounds through the use of computational techniques, network analysis, and the integration of omics data [11]. Furthermore, the predictive power of this method helps prioritize target proteins and candidate compounds for subsequent experimental validation, thereby accelerating the drug development process [12-14]. Additionally, molecular docking and molecular dynamics simulations were employed to clarify the binding interactions between bitter leaf components and their specific targets, thereby offering a more comprehensive understanding of their pharmacological effects. Therefore, this study aimed to investigate the molecular mechanisms underlying the anti-breast cancer activity of *Vernonia amygdalina* using network pharmacology and molecular docking approaches

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Biological Materials

Vernonia amygdalina phytochemicals

2.1.2 Chemicals and Reagents

None (computational study)

2.1.3 Equipment and other materials

ADMETlab, SwissTargetPrediction, STRING, Cytoscape, GEPIA2, PyRx, Discovery Studio and UCSF ChimeraX

2.2 METHODS

2.2.1 Data acquisition, ADME analysis, and component screening of Vernonia amygdalina (VA)

The Phyto molecules reported in VA were retrieved from the Phytochemical Interaction Database (https://www.genome.jp/db/pcidb/). After removing duplicate entries, the compounds were screened for ADME and pharmacokinetics using the ADMETlab2.0 online platform (https://admetmesh.scbdd.com/) [15]. This platform provides a comprehensive evaluation and scoring system of the physicochemical properties, pharmacokinetics, and medicinal chemistry of potential drug candidates [15]. The canonical SMILES structures, molecular formulas, and

three-dimensional conformations of the compounds were downloaded from PubChem and used for subsequent computational analyses [16]. The canonical SMILES of each compound were entered into the SwissTargetPrediction (STP) platform (http://www.swisstargetprediction.ch/) in order to molecularly predict potential human protein targets and host-associated molecular interaction concerning the Breast Cancer [17]. SwissTargetPrediction finds potential macromolecular targets as a result of a 2D and 3D chemical similarity search of known ligands that have been successfully tested. To be more precise, the targeted proteins were limited to *Homo sapiens* so that its relevance to the host interaction pathways implicated in the replication, immune modulation, and inflammatory signaling during infection with breast cancer can be achieved. Based on the data obtained, the analysed compounds were screened on four diverse characteristics: positive for Lipinski's rule, human oral bioavailability (F30%) (threshold value < 0.3), ability to penetrate the blood-brain barrier (BBB) (threshold value < 0.3), and QED drug-likeness score (threshold value > 0.67). The identified targets were subsequently curated and employed in downstream analyses such as protein-protein interaction mapping, pathway enrichment and molecular docking studies in order to explain the possible mechanisms by VA can suppress the activity of breast cancer pathways. These selected compounds were then used for further analysis.

2.2.2 Data collection of Breast Cancer–associated genes

Additional Breast cancer–related genes were systematically retrieved from the Gene Expression Profiling Interactive Analysis (GEPIA 2) database (http://gepia2.cancer-pku.cn/), which integrates genomic, transcriptomic, proteomic, and functional annotations for human genes. This resource provides a comprehensive repository of gene disease associations and biological pathway information relevant to cancer diseases.[18].

2.2.3 Preparation of ligands and target prediction

The chemical structures of the phytochemicals namely Vermogdylin, Hydroxyvernolide, Vernodaline, 11,13-Dihydrovernodaline, and Vernolide were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), which provides curated chemical structures, physicochemical descriptors, and biological activity information for small molecules [16]. The canonical SMILES structures and molecular descriptors were retrieved and used for computational target prediction. The SMILES structures of the compounds were submitted to the SwissTargetPrediction (STP) platform (http://www.swisstargetprediction.ch/) to predict potential human protein targets. STP estimates probable molecular targets of bioactive small molecules by integrating 2D and 3D chemical similarity metrics with known ligand–target interactions [17]. The organism parameter was restricted to *Homo sapiens* to ensure relevance to host molecular pathways associated with Breast cancer. Predicted targets were collected and curated by removing duplicates and converting identifiers into official Homo sapiens gene symbols. The predicted protein targets were then compared with the curated Breast cancer–associated gene dataset to identify overlapping genes potentially involved in antiviral activity. The overlapping genes were identified using the Venny 2.1.0 platform. The compound target interaction network was subsequently constructed and visualized using Cytoscape (version 3.10.4), which allows integrated visualization and analysis of biomolecular interaction networks [18].

2.2.4 Overlapping Targets for VA Targets and Breast cancer

On the other hand, VENNY2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) [19] was used to recognize and demonstrate the shared targets that overlapped between VA phytochemicals and Breast cancer. The relationship between VA phytochemicals and their targets is revealed by the intersection of the identified targets.

2.2.5 Creating a Network Involving Intersecting Targets

Protein-protein interaction between phytochemicals targeted genes related to Breast Cancer (BC) was retrieved using the STRING database Version 12.0 (https://string-db.org/) [20] with *Homo sapiens* mode and a confidence level of >0.7. After that, the network was analysed using the Cytoscape 3.10.4 (https://cytoscape.org/) software program [21], and the CytoHubba module within Cytoscape was utilized to identify essential genes.

2.2.6 Drugs, Targets, and Pathways (Ds-T-P) Network

Construction and Analysis Ds-T-P network was constructed using Cytoscape's merger algorithm plugin (Cytoscape) [18]. With the help of a network analyzer, the network topology is analyzed. Nodes represented drugs, targets, and pathways, and interactions between these nodes were shown along the edges. The frequency of a node's connected neighbors was also indicated by the degree. The more significant the influence, the higher the fraction of nodes that are directly connected.

2.2.7. Gene Ontology (GO) Enrichment and KEGG Pathways Analysis of Common Intersected Targets

Using ShinyGO v0.85 (http://bioinformatics.sdstate.edu/go/) [22], functional enrichment analysis of the common intersected targets was initially carried out using Homo sapiens as the chosen species. To find statistically significant GO keywords and KEGG pathways, such as molecular function (MF), cellular components (CC), and biological process (BP) categories, a false discovery rate (FDR) criterion of 0.05 was used. This made it possible to systematically identify canonical signalling pathways and molecular activities linked to the intersected targets. Gene Ontology (GO) and KEGG pathway interpretation were then used to determine how each intersected target functions in signalling pathways. The official gene symbol and Homo sapiens as a species were used for this enrichment study for GO term characterization.

2.2.8. Molecular Docking

Molecular docking studies were carried out using PyRx 0.8 to elucidate the interactions between targets and BC associated protein targets. The three-dimensional (3D) structures of the phytochemicals were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and imported into PyRx, where they were energy-minimized using the Universal Force Field (UFF) prior to docking. The crystallographic structures Breast Cancer Aurora Kinase A (AURKA, PDB ID: 5DOS) [23] and Thymidylate Synthase (TYMS, PDB ID: 6OJU) [24], were identified through extensive literature mining and obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) [25]. Multiple docking runs were performed for each ligand–protein system, and binding affinities were reported in kcal/mol. The minimum binding energy and advantageous interaction geometry were used to determine which binding poses were ranked highest. BIOVIA Discovery Studio Visualiser 2025 was used to visualise and conduct a thorough interaction analysis of the docked complexes, including hydrogen bonding, hydrophobic interactions, and electrostatic contacts.

2.3 Statistical Analysis

Statistical significance for GO and KEGG enrichment analyses was determined using a false discovery rate (FDR) threshold of <0.05. Protein-protein interaction enrichment significance was assessed using STRING database statistical algorithms. Descriptive analyses were performed using Cytoscape and ShinyGO platforms.

3.0 RESULTS

3.1. Drug-Likeness Filtering and Selection of Bioactive Drug candidate

Based on the drug-likeness and pharmacokinetic characteristics of the identified phytochemicals compounds against Breast Cancer were filtered to identify bioactive and pharmacologically relevant molecules. The screening criteria were based on molecular weight (≤500 Da), hydrogen bond donors (HBD ≤5), hydrogen bond acceptors (HBA ≤10), lipophilicity (LogP ≤5), topological polar surface area (TPSA ≤140 Å²), absence of pan-assay interference compounds (PAINS), and compliance with Lipinski's rule of five, as implemented in the ADME/T Lab 3.0 platform (https://admetlab3.scbdd.com/) [26]. Among the initially identified phytochemicals, twelve compounds satisfied the drug-likeness thresholds without violating critical pharmacokinetic parameters (Table 1 and 2). The prediction of the absorption, distribution, metabolism, excretion (ADME), and toxicity profiles of the chosen repurposed antiviral compounds was done through the ADMETlab 3.0 web platform. It is necessary that a drug molecule crosses intestinal epithelium by passive diffusion or active transport mechanism before entering the systemic circulation. The Caco-2 cells, which were obtained by dissection of human colon adenocarcinoma, represent a common in vitro model of the human intestinal barrier to determine the intestinal permeability of drug candidates, as they replicate the morphological and transport properties of intestinal epithelial cells [15]. The pharmacokinetic and toxicity profiles of the selected phytochemicals were evaluated to determine their drug-likeness, absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties (Table 2). The compounds investigated included 11,13-Dihydrovernodalin (1), Hydroxyvernolide (2), Vernodaline (3), Vernolide (4), and Vernomygdin (5). Regarding absorption properties, all compounds demonstrated moderate Caco-2 permeability values ranging from −5.22 to −4.85, suggesting acceptable intestinal permeability. Hydroxyvernolide (2) and Vernolide (4) exhibited relatively higher human intestinal absorption (HIA) values of 0.56 and 0.47, respectively, indicating improved oral absorption potential compared with the other compounds. Vernomygdin (5) showed the lowest HIA value (0.064), suggesting comparatively reduced gastrointestinal absorption. P-glycoprotein (P-gp) inhibition analysis revealed low-to-moderate inhibitory tendencies among the compounds, although Vernomygdin (5) displayed the highest P-gp inhibition probability (0.80), indicating a greater likelihood of influencing drug efflux transport mechanisms. For distribution characteristics, all

phytochemicals demonstrated low blood–brain barrier (BBB) permeability values, ranging from 0.0012 to 0.090, indicating limited penetration into the central nervous system. Plasma protein binding analysis revealed moderate-to-high binding affinities, with Vernolide (4) exhibiting the highest plasma protein binding value (79.4%), suggesting prolonged systemic circulation and reduced free-drug concentration in plasma. Metabolic profiling against cytochrome P450 isoenzymes showed generally low inhibition tendencies across CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 enzymes. This observation suggests a reduced probability of cytochrome-mediated drug–drug interactions. However, Vernolide (4) exhibited comparatively higher inhibitory values against CYP1A2 (0.17), CYP2C9 (0.14), and CYP3A4 (0.043), indicating a relatively greater interaction potential than the other phytochemicals. Vernomygdin (5) also demonstrated a notably elevated CYP3A4 inhibition probability (0.79), suggesting possible metabolic interaction liabilities. Excretion analysis demonstrated moderate human clearance values for all compounds, ranging from 3.97 to 4.65. Vernomygdlin (5) showed the highest clearance value (4.65), while 11,13-Dihydrovernodalin (1) exhibited the lowest (3.97). Predicted half-life values ranged from 1.53 to 2.05, with Vernolide (4) demonstrating the longest half-life, indicating relatively prolonged systemic persistence. Toxicological assessment revealed generally favorable safety profiles for the investigated phytochemicals. All compounds exhibited low hERG blocking probabilities (0.0048–0.026), suggesting minimal risk of cardiotoxicity associated with QT interval prolongation. Mutagenicity and tumorigenicity predictions were negative for all compounds, indicating the absence of mutagenic and tumorigenic tendencies. However, relatively high probabilities of drug-induced liver injury (DILI) were observed, particularly for Vernodaline (3) and Vernomygdin (5), with values of 0.97 and 0.94, respectively, suggesting potential hepatotoxicity concerns that may require further experimental validation. Similarly, carcinogenicity predictions varied among the compounds, with Vernodaline (3) showing the highest carcinogenicity probability (0.95), whereas Vernolide (4) demonstrated the lowest value (0.15). Ames toxicity predictions indicated moderate probabilities across the compounds, ranging from 0.59 to 0.93. Additionally, all compounds demonstrated positive skin sensitization outcomes. LC50 values ranged from 4.86 to 5.28, indicating moderate toxicity levels among the phytochemicals. Overall, the findings indicate that the investigated phytochemicals possess favorable pharmacodynamic and pharmacokinetic characteristics, supporting their potential as multi-target therapeutic candidates against breast cancer.

Table 1: Physicochemical properties of the compounds

Peak ID	MW	Fraction sp3	LogP	LogS	Natural Product-likeness	TPSA	nHA	nHD	ROA	Lipinski violation	PAINS
1	362.14	0.526	0.570	-1.928	3.252	99.13	7	1	0.147	0	0
2	378.13	0.579	0.506	-1.912	3.643	114.82	8	2	0.244	0	0
3	360.12	0.421	0.809	-2.278	3.163	99.13	7	1	0.206	0	0
4	362.14	0.579	1.226	-2.737	3.519	94.59	7	1	0.376	0	0
5	364.15	0.341	1.593	-2.477	3.393	94.59	7	1	0.839	0	0

Key: MW: Molecular weight, TPSA: topological polar surface area. iLogP: logarithm of noctanol-water partition coefficient. of a compound). nHA (Number of Hydrogen Bond Acceptors), nHD (Number of Hydrogen Bond Donors), ROA (Rotatable Bonds or Rotatable Bond Count), and PAINS: pan assay interaction structures. Note: 1: represents: 11,13-Dihydrovernodalin; 2: Hydroxyvernolide 3: Vernodaline ; 4; Vernolide; 5: Vernomygdin

Table 2: Results of Pharmacodynamics and Pharmacokinetics potentials of phytochemicals of V. amygdalina
DILI: drug-induced liver injury, hERG: human ether a-go-go

	Properties	1	2	3	4	5
Absorption	Caco-2 permeability	-5.22	-4.87	-4.85	-4.85	-4.92
	HIA	0.24	0.56	0.34	0.47	0.064
	P-gp inhibition	0.31	0.22	0.055	0.39	0.80
Distribution	BBB Permeability	0.0073	0.0012	0.0044	0.0037	0.090
	Plasma protein Binding (Human)	65.6%	54.8%	65.6%	79.4%	67.4%
Metabolism	CYP 1A2 Inhibition	0.00010	0.0056	0.00000049	0.17	0.00037
	CYP 2C19 Inhibition	0.0000011 6	0.00013	0.0000012	0.027	0.0071

	CYP 2C9 inhibition	0.00011	0.0025	0.00010	0.14	0.009
	CYP 2D6 inhibition	0.0000035	0.0016	0.00000097	0.018	0.0019
	CYP 3A4 inhibition	0.056	0.00091	0.00061	0.043	0.79
Excretion	Human clearance	3.97	4.46	4.29	4.55	4.65
	Half life	1.67	1.93	1.64	2.05	1.53
Toxicity	hERG blocker	0.0048	0.014	0.0071	0.026	0.019
	Drug-induced liver injury	0.89	0.79	0.97	0.80	0.94
	Mutagenecity	0	0	0	0	0
	Carcinogenicity	0.89	0.19	0.95	0.15	0.49
	Tumorigenicity	0	0	0	0	0
	LD50_oral	0	0	0	0	0
	Ames	0.74	0.64	0.86	0.59	0.93
	LC50	5.17	4.98	5.28	5.005	4.86
	Skin Sensitization	1	1	1	1	1

Note: 1: represents: 11,13-Dihydrovernodalin; 2: Hydroxyvernolide 3: Vernodaline ; 4; Vernolide; 5: Vernomygdin

3.2. Common Intersected Targets of Repurpose Drugs within the Pubchem and STP Database

Following ADME screening, the target prediction for 5 compounds was carried out using the pubchem databases. Simultaneously, the SMILES code for every phytochemical compound was input into the search queries. The number of targets from the pubchem databases was calculated in venny 2.0.1 with breast cancer genes (BRCA_genes) 721 (73.6%) and phytochemicals target to be 238 (24.3%), respectively in accordance to the Venn diagram analysis, 21 (2.1%) common targets between these two databases were found as overlaps (Fig. 1). The 2D structures of V. amygdalina phytochemicals were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Genes associated with breast cancer were retrieved from the Gepia 2 database (http://gepia2.cancer-pku.cn/) were predicted using SwissTargetPrediction (https://www.swiss targetprediction.ch). A total of 21 (2.1%) target genes were found to overlap when the target genes linked to phyto compounds related overlapping breast cancer genes were investigated, and it is depicted in Fig. (1).

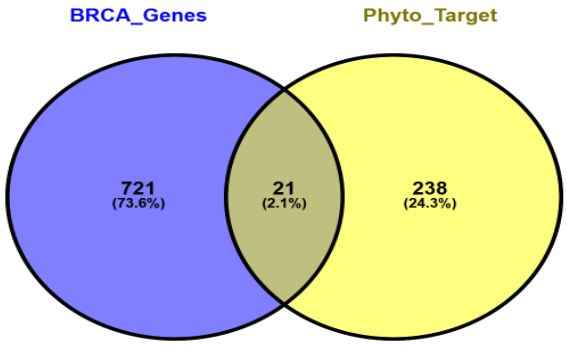


Figure 1: Overlap of predicted molecular targets of VA with Breast cancer associated-genes, highlighting common therapeutic targets.

3.3 Protein–protein interaction (PPI) network analysis

The intersected gene set was imported into STRING v12.0 (https://string-db.org/) with Homo sapiens as the reference organism and a high confidence interaction threshold (≥0.97). The resulting PPI network comprised 21 nodes and 39 edges, with an average node degree of 3.71 and an average local clustering coefficient of 0.483, indicating a highly interconnected network structure. The expected number of edges was 15, while the observed number of interactions

was substantially higher, yielding a PPI enrichment p-value is $9.18 \times 10^{-}08$, which demonstrates that the network contained significantly more interactions than would be expected by chance (Fig. 2A). Hub genes were identified based on degree and betweenness centrality parameters. The top 10 hub proteins included AURKA, TYMS, EGFR, TK1, CDK1, TOP2A, AURKB, KIT, KIF11 and MMP1 (Fig. 2B). PPI networks were visualized using Cytoscape v3.10.3. With the help of the network analyser tool from Cytoscape, the topological parameters of the network are analysed. We categorized the elements in the network according to their degree values. Based on degree value, AURKA and TYMS were selected as major proteins that may have a significant therapeutic effect on breast cancer.

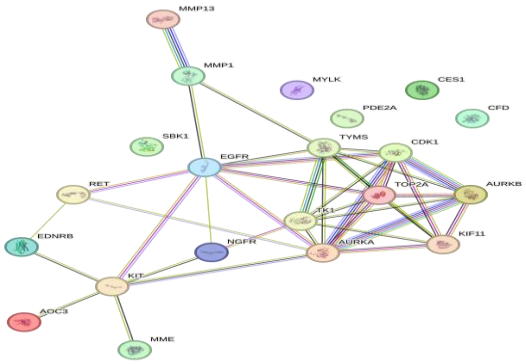


Fig 2A: Protein–protein interaction (PPI) network of the overlapping targets, revealing key hub proteins involved in breast cancer disease pathology.

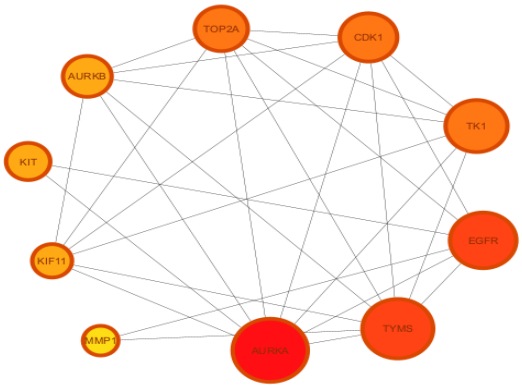


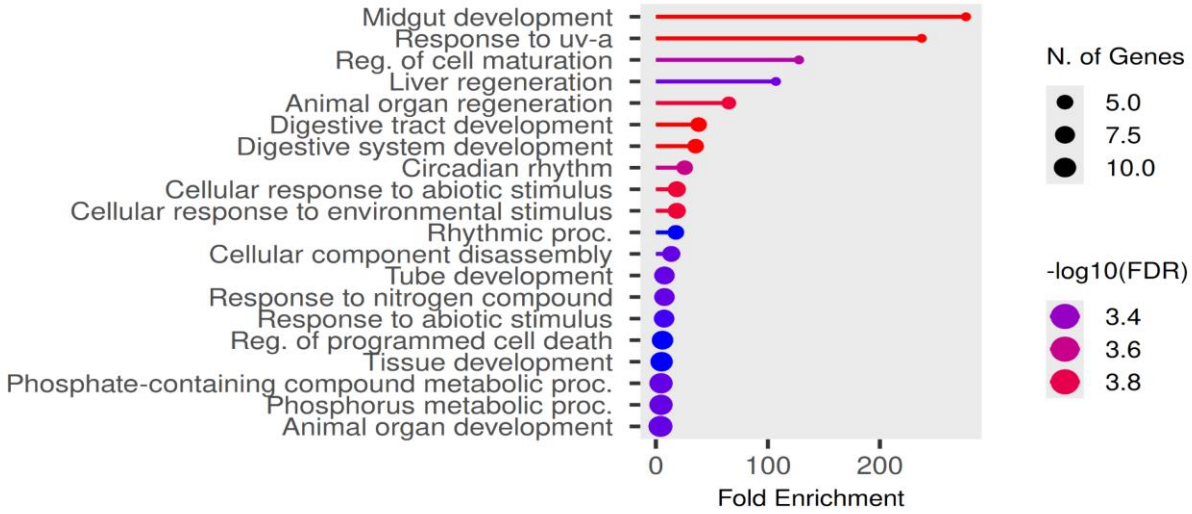
Figure 2B. Top ten most significant overlapping genes ranked as core targets of V. amygdalina phytochemicals with breast cancer-genes, highlighting common therapeutic targets.

3.4. Gene-Ontology (GO) and KEGG Pathway Enrichment Analysis of Common Targets

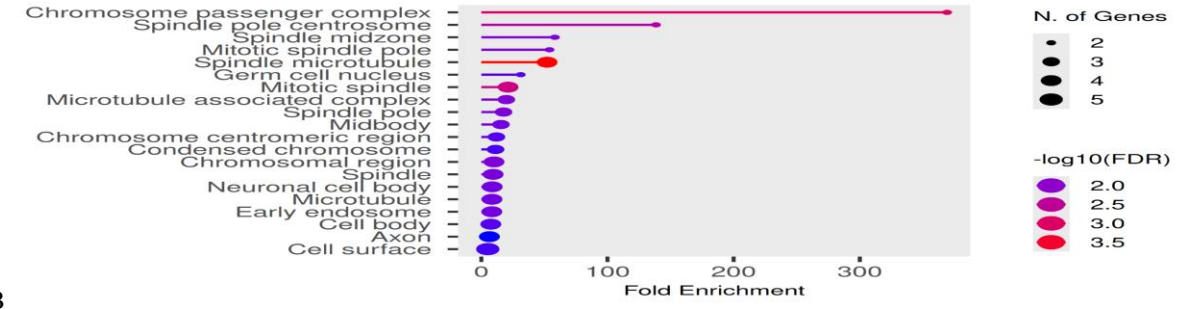
We used a web-based tool, ShinyGO, to analyse Gene Ontology (GO) and KEGG pathways for the predicted targets associated with the proteins AURKA and TYMS against Breast Cancer. Based on the proportion, we identified the top 10 Molecular Functions (MF), Biological Processes (BP), and Cellular Components (CC). Several biological processes have been linked to breast cancer progression and survival as illustrated in Fig 3A. Midgut development, digestive tract development, and digestive system development are associated with cancer because tumor cells often reactivate embryonic growth pathways to support uncontrolled growth and metastasis [29]. Response to UV-A and cellular response to abiotic or environmental stimuli may contribute to DNA damage and mutation accumulation involved in breast cancer development [30]. Animal organ regeneration, regulation of cell maturation, and liver regeneration are connected with abnormal cell proliferation and tissue remodeling seen in tumors [31]. In addition, disruption of the circadian rhythm has been associated with increased breast cancer risk due to hormonal imbalance and impaired cellular repair mechanisms [30]. Cellular components' targets as illustrated in Fig 3B include spindle microtubule, chromosome passenger complex, mitotic spindle, spindle pole centrosome, spindle pole, microtubule-associated complex, spindle midzone, mitotic spindle pole, chromosomal region, and spindle are mainly involved in cell division and chromosome separation. Their enrichment in breast cancer suggests increased cell proliferation and

uncontrolled tumor growth, which are common features of cancer progression. Abnormal regulation of these spindle-related structures can lead to chromosome instability, mutations, and metastasis in breast cancer cells [29]. These findings indicate that many predicted targets are associated with intracellular structures that regulate mitosis, cell-cycle progression, and cancer cell survival. The identified molecular functions shown in Fig 3C including adenyl ribonucleotide binding, anion binding, adenyl nucleotide binding, kinase activity, protein kinase activity, ATP binding, ribonucleotide binding, purine ribonucleotide binding, heterocyclic compound binding, and purine nucleotide binding, are closely associated with breast cancer development and progression. Many of these functions are involved in energy transfer, signal transduction, and regulation of cell growth. In particular, kinase and protein kinase activities play important roles in cancer cell proliferation, survival, metastasis, and resistance to therapy because they control major signaling pathways involved in tumor progression [29]. ATP- and nucleotide-binding activities are also essential for rapid cancer cell metabolism and continuous cell division observed in breast cancer cells [32]. Similarly, the top 20 KEGG pathways illustrated in Fig 3D and 3E were identified at a threshold level of P-value < 0.001 for the predicted therapeutic targets associated with breast cancer. Important KEGG pathways include Relaxin signaling pathway, central carbon metabolism in cancer, calcium signaling pathway, pathways in cancer, MAPK signaling pathway, PI3K-Akt signaling pathway, cGMP-PKG signaling pathway, bladder cancer, Rap1 signaling pathway, pyrimidine metabolism, Ras signaling pathway, Coronavirus disease (COVID-19), non-small cell lung cancer, drug metabolism-other enzymes, nucleotide metabolism, IL-17 signaling pathway, hematopoietic cell lineage, melanogenesis, progesterone-mediated oocyte maturation, and parathyroid hormone synthesis, secretion and action. These enriched pathways are mainly involved in cancer cell proliferation, metabolism, inflammation, angiogenesis, immune regulation, and metastasis. In particular, the MAPK, PI3K-Akt, Ras, and IL-17 signaling pathways are strongly linked to breast cancer progression, tumor survival, and resistance to therapy. Therefore, these pathways suggest that the identified therapeutic compounds may exert anticancer effects by modulating multiple signaling and metabolic pathways involved in breast cancer development and progression [33].

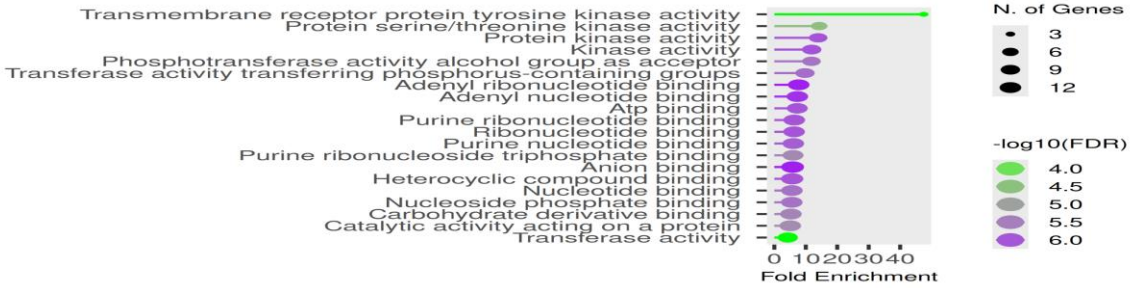
A



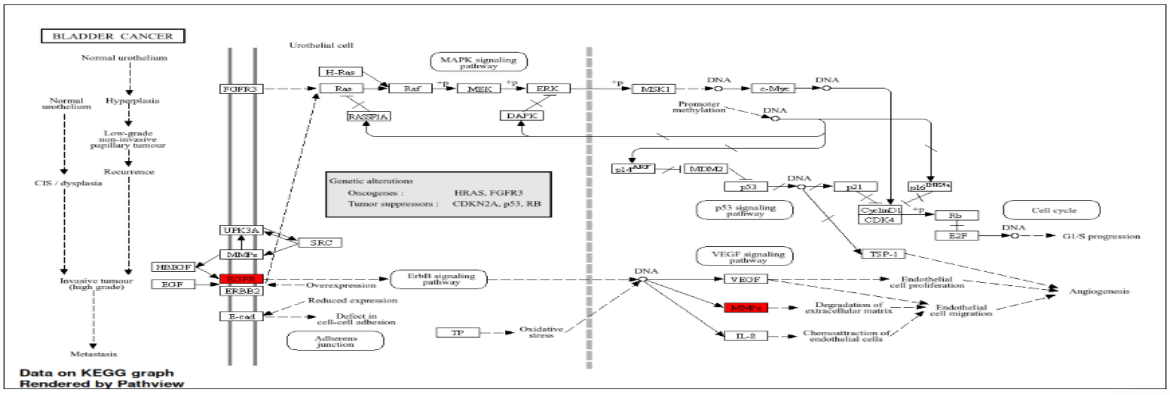
B



C



D



E

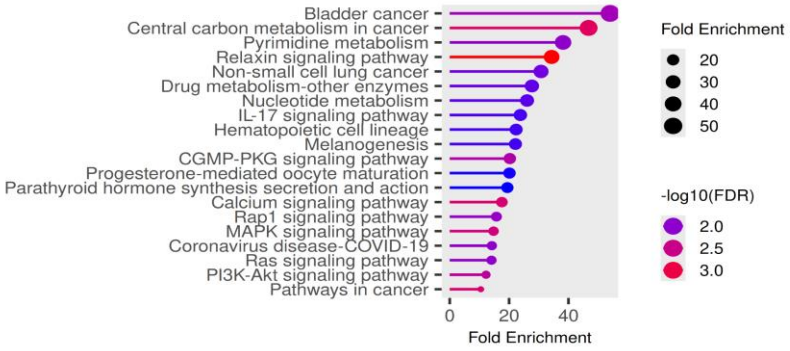


Fig 3: GO analysis, in the top 20 significantly enriched terms, the y-axis represents the enriched category, the x-axis represents the number of enrichments and the order of importance was ranked from top to bottom by − Log10 (p value). A = Biological process. B = Cellular component. C =Molecular function. KEGG analysis. 3D: pathway enrichment of the overlapping targets, indicating significantly modulated inflammation-related signaling pathways. 3E: Pathway maps of potential targets for KEGG analysis pathway with significance value at $p < 0.05$. The red nodes represent the targets of V amygdalina.

3.5 Molecular docking studies

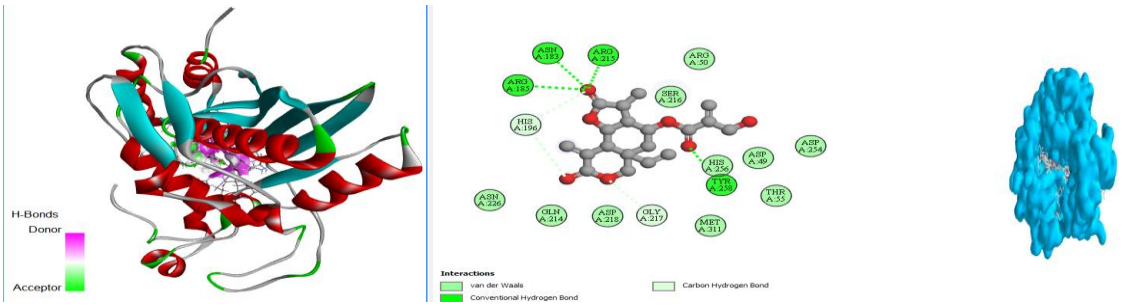
Based on the results of network pharmacology and target prediction, molecular docking was performed to elucidate the interactions between *V. amygdalina* phytochemicals and the key breast cancer gene target, Aurora Kinase A (AUKRA; PDB ID: 5DOS; method: X-ray diffraction; resolution: 2.98 Å) and Thymidylate Synthase (TYMS; PDB ID: 6OJU; method: X-ray diffraction; resolution: 2.884 Å), which are implicated in breast cancer. The two-dimensional (2D) and three-dimensional (3D) structure data files (SDF) of the phytochemicals were retrieved from the PubChem database. Ligand structures were prepared and initially optimized using BIOVIA Discovery Studio Visualizer 2025, followed by conversion into PDB format. The crystal structure of AUKRA and TYMS were downloaded from the Protein Data Bank (https://www.rcsb.org/). Protein preparation was carried out using UCSF ChimeraX (2025 release), including removal of crystallographic water molecules and co-crystallized ligands, correction of missing residues, and addition of polar hydrogens. The prepared protein structure was energy minimized

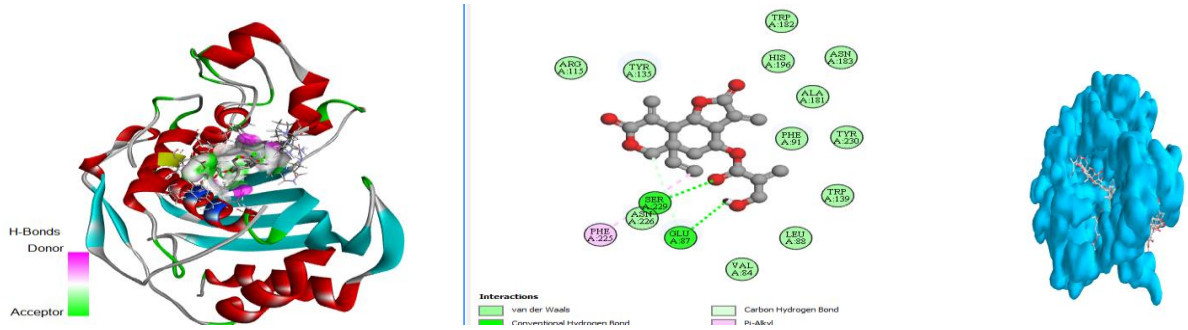
using the AMBER ff14SB force field to obtain a stable conformation prior to docking. Prepared ligands and receptor structures were imported into PyRx 0.8, where they were converted into PDBQT format compatible with AutoDock Vina. Ligand energy minimization was performed in PyRx using the Universal Force Field (UFF) solely to relieve geometric strain and generate reasonable starting conformations prior to docking. The docking grid was centered on the active binding region of AUKRA (grid center: X = − 10.8234, Y = − 32.9905, Z = 8.5683) and TYMS (grid center: X = 30.5608, Y = 36.4834, Z = −17.3172), with grid box dimensions optimized to encompass the binding pocket. Docking simulations were conducted using AutoDock Vina with a default exhaustiveness value of eight to ensure adequate conformational sampling. Binding affinities were reported in kcal/mol, and the top-ranked binding poses were saved as PDB files. The resulting protein–ligand complexes were further analyzed and visualized using BIOVIA Discovery Studio Visualizer 2025 and UCSF ChimeraX to assess key molecular interactions. Hydrogen bonding, hydrophobic interactions, π–π stacking, and electrostatic contacts between the phytochemicals and AUKRA, TYMS, and EGFR active-site residues were identified to evaluate binding complementarity and stability. Docking scores and detailed amino acid interactions are presented in Table 3, while representative 2D and 3D binding modes are illustrated in Fig. 4. The docking protocol and analytical procedures followed previously validated methodologies described] by Saddala and Huang [34] and Kim et al. [35].

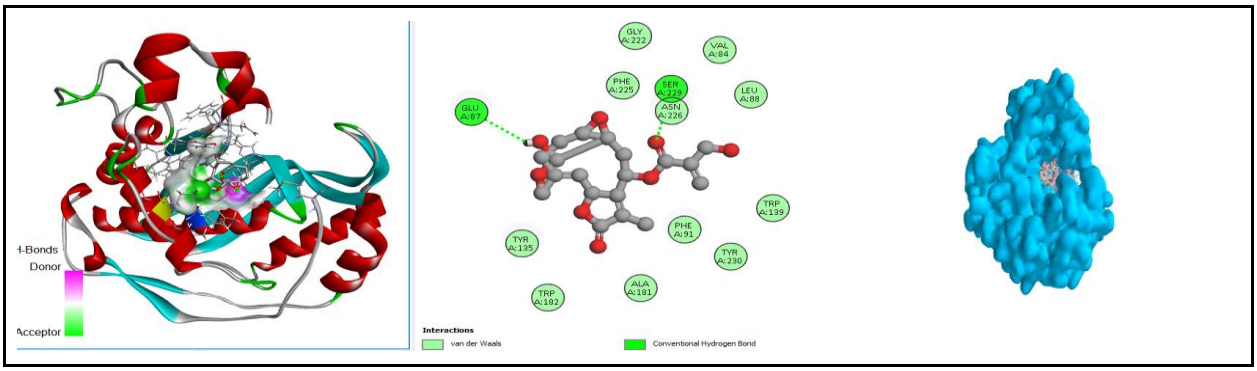
Table 3. Docking scores (kcal/mol) of the breast cancer proteins, TYMS and AUKRA with key phytochemical targets.

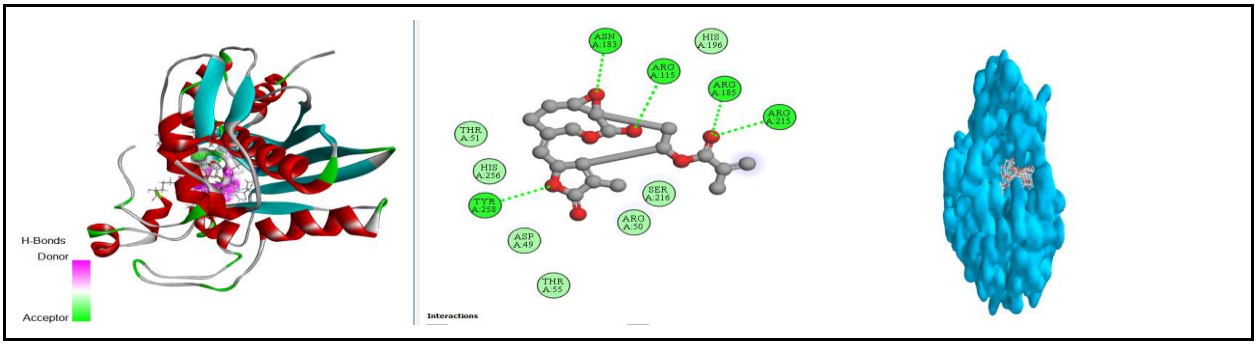
S/NO	Phytochemical	Docking score of TYMS	Docking score of AUKRA	Docking score of EGFR
	11,13-dihydrovernodaline	-8.1	-8.0	-7.7
	Vernodalin	-8.1	-7.1	-7.8
	Hydroxyvernolide	-8.6	-6.6	-7.8
	Vernolide	-8.0	-7.1	-7.6
	Vernomygdin	-8.6	-6.3	-7.5

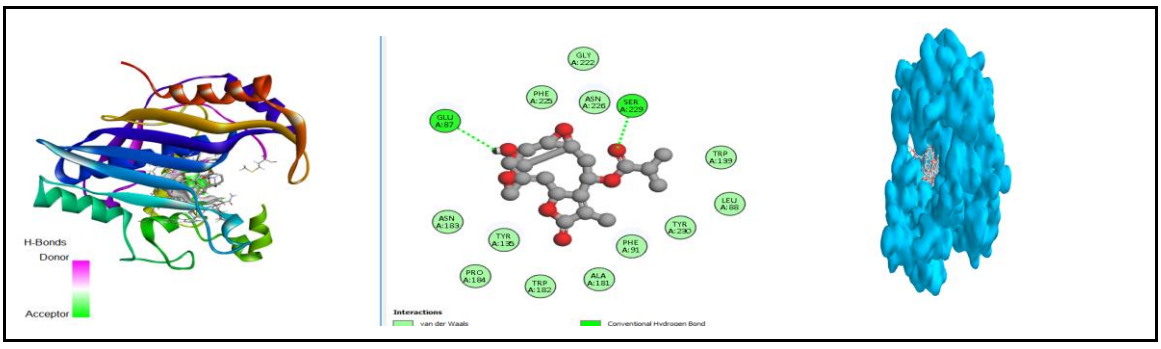
A-TYMS




H-Bonds
Donor
Acceptor
Interactions
van der Waals
Carbon Hydrogen Bond

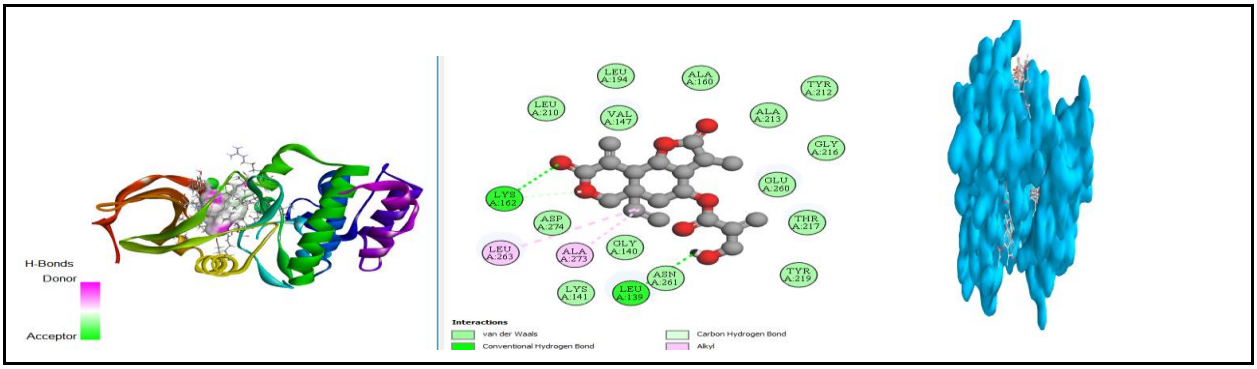

Donor
Acceptor
Interactions
van der Waals
Conventional Hydrogen Bond


H-Bonds
Donor
Acceptor
Interactions

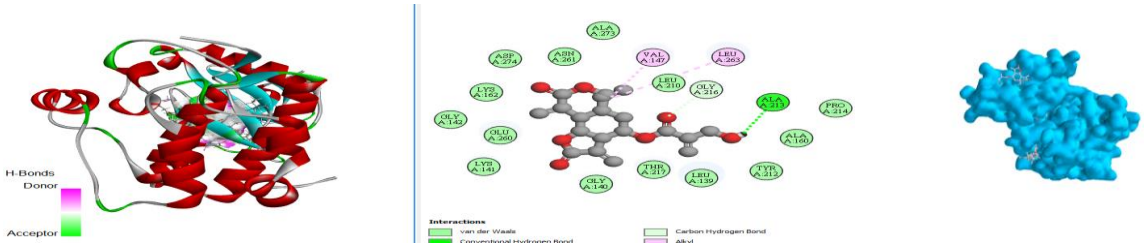

H-Bonds
Donor
Acceptor
Interactions
van der Waals
Conventional Hydrogen Bond

B-AUKRA

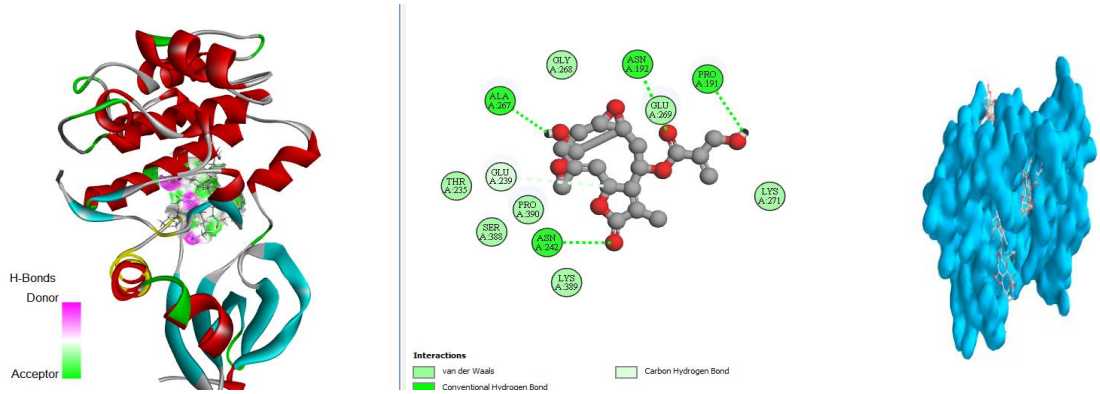
1.


H-Bonds
Donor
Acceptor
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl

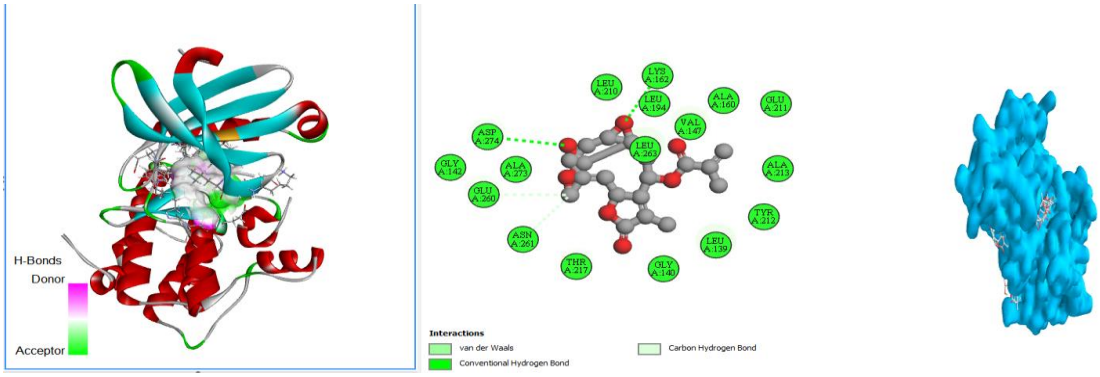
2.


H-Bonds
Donor
Acceptor
Interactions

3.


H-Bonds
Donor
Acceptor
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond

4.


H-Bonds
Donor
Acceptor
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond

5.

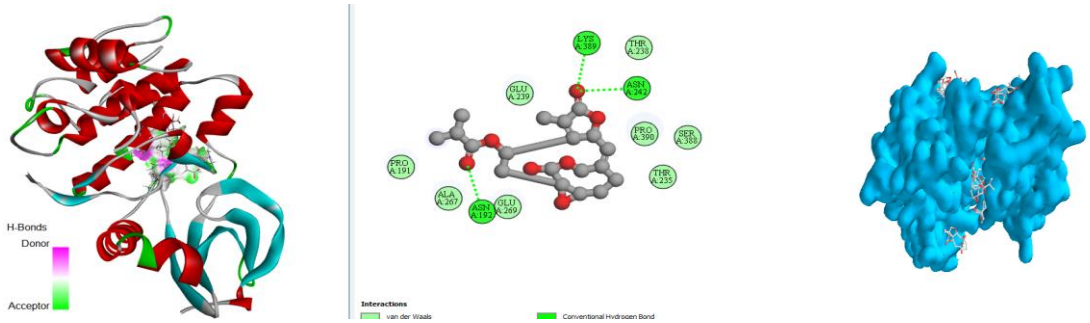


Fig 4: Molecular docking visualizations of the five compounds of *V. amygdalina* with target protein of TYMS (PDB ID: 6OJU) and AUKRA (PDB ID: 5DOS) using Discovery Studio. The visualization on the left shows the protein structure and its hydrophobicity, and the center picture reveals the 2D interaction of target-ligand complexes while that of the right shows the target protein with its ligand at the binding pocket. The compounds are 11,13-Dihydrovernodalin, Hydroxyvernolide, Vernodalin, Vernolide, and Vernomygdin. Note: 1: represents: 11,13-Dihydrovernodalin; 2: Vernodalin; 3: Hydroxyvernolide 4; Vernolide; 5: Vernomygdin

4. DISCUSSIONS.

The current study used an integrated network pharmacology and molecular docking technique to clarify the molecular mechanisms behind Vernonia amygdalina's anti-breast cancer efficacy. Our results imply that *V. amygdalina's* anticancer effects are mediated by a multi-component, multi-target, and multi-pathway mechanism involving important regulators of metabolism, survival signaling, cell proliferation, and cell-cycle progression. This systems-level approach is especially pertinent to breast cancer, which is a very heterogeneous illness that is not caused by a single genetic aberration but rather by dysregulation of interconnected signaling networks. Five main phytochemicals with favorable physicochemical and pharmacokinetic properties were found in the initial ADMET and drug-likeness screening: 11,13-dihydrovernodalin, hydroxyvernolide, vernodalin, vernolide, and vernomygdin. Lipinski's rule of five, adequate intestinal absorption, low blood–brain barrier permeability, and minimal projected cytochrome P450 inhibition were among the primary drug-likeness requirements that these substances met. Oral bioavailability and the success of drug development are significantly influenced by these characteristics [36, 37]. The overall pharmacokinetic profile supports the compounds' potential as lead molecules for additional preclinical testing, despite the fact that some of them showed increased probabilities of drug-induced liver injury. Sesquiterpene lactones extracted from *V. amygdalina* have been shown to exhibit promising anticancer properties, although more toxicological confirmation is necessary [38, 39]. 21 overlapping targets between V. amygdalina phytochemicals and genes linked to breast cancer were found using network pharmacology analysis, underscoring the plant's polypharmacological characteristics. Multi-target medicines, in contrast to traditional single-target therapies, can concurrently affect many oncogenic pathways, potentially lowering the risk of therapeutic resistance. This finding is consistent with the increasing understanding that natural products do not work by a single molecular interaction, but rather by coordinated regulation of intricate biological networks [40,41]. Further study of protein-protein interactions identified TYMS and AURKA as the network's most important hub genes. Serine/threonine kinase Aurora kinase A (AURKA) is essential for chromosomal segregation, centrosome maturation, mitotic spindle construction, and cell-cycle progression. Chromosome instability, tumour aggressiveness, poor prognosis, and treatment resistance have all been linked to aberrant overexpression of AURKA in breast cancer [42,43]. As a result, inhibiting AURKA is a viable method for stopping unchecked growth and causing mitotic catastrophe in breast cancer cells. In a similar vein, thymidylate synthase (TYMS) is an essential enzyme for nucleotide metabolism and de novo DNA synthesis. Rapid tumour growth is encouraged by elevated TYMS expression, which has also been linked to a poor response to a number of chemotherapeutic drugs, especially fluoropyrimidine-based treatments [44]. This study's identification of TYMS as a critical hub target raises the possibility that *V. amygdalina* phytochemicals may impede cellular proliferation and DNA replication, hence slowing the growth of tumours. Targeting AURKA and TYMS at the same time is especially significant because these proteins control related processes cell division and DNA biosynthesis that are necessary for cancer cell survival. The selected targets' participation in basic cancer-related processes was further corroborated by Gene Ontology enrichment analysis. *V. amygdalina* may have therapeutic effects beyond direct cytotoxicity, as evidenced by the enrichment of biological processes linked to cellular development, tissue

regeneration, environmental sensitivity, and circadian rhythm. By affecting cellular proliferation, DNA repair, and hormonal signaling, dysregulated developmental programs and circadian rhythm disruptions are linked to the development and progression of breast cancer, according to mounting evidence [45]. Moreover, the preponderance of cellular elements involved with chromosomal passenger complexes, microtubule-associated structures, and mitotic spindle architecture highlights the significance of cell-cycle regulation among the anticipated targets. Chromosome instability, a characteristic of cancer that propels tumour development and spreading potential, is influenced by defects in these structures [46]. Significant correlations between kinase activity, protein kinase activity, ATP binding, and nucleotide-binding functions were found in the molecular function enrichment data. Because they affect signaling pathways that govern proliferation, apoptosis, angiogenesis, and metastasis, protein kinases are among the most significant therapeutic targets in oncology [47]. The multitarget pharmacological profile found in this work is consistent with the enrichment of kinase-related functions, which implies that *V. amygdalina* phytochemicals may have wide regulatory effects on oncogenic signaling cascades. KEGG pathway analysis provides additional mechanistic insight by finding several well-established cancer pathways, including PI3K-Akt, MAPK, Ras, Rap1, IL-17, calcium signaling, and central carbon metabolism in cancer. One of the most commonly dysregulated pathways in breast cancer is the PI3K-Akt pathway, which encourages tumour growth, survival, metabolic adaptation, and treatment resistance [48]. Similarly, unchecked growth and metastatic spread are caused by abnormal MAPK and Ras signaling. These pathways' enrichment indicates that *V. amygdalina* may have anticancer effects by coordinating the inhibition of several oncogenic signaling networks rather than just one. In the treatment of breast cancer, such multi-pathway modulation is increasingly recognized as a beneficial tactic for overcoming drug resistance. The network pharmacology predictions were further confirmed by molecular docking studies. Strong binding affinities for TYMS and AURKA were shown by the chosen phytochemicals; hydroxyvernolide and vernomygdin showed the strongest interactions with TYMS (−8.6 kcal/mol), while 11,13-dihydrovernodalin showed the highest affinity toward AURKA (−8.0 kcal/mol). The potential for direct inhibitory action against these proteins is supported by these binding energies, which show advantageous ligand–target interactions. Sesquiterpene lactones from *V. amygdalina*, such as vernodalin and vernolide, have been shown in earlier research to have strong cytotoxic and antiproliferative effects against a number of cancer cell lines by inducing apoptosis and suppressing PI3K/Akt and MAPK signaling pathways [38, 39]. As a result, our docking data offer mechanistic support for current experimental findings and bolsters the need for additional biological validation. The study's overall results provide credence to the theory that V. amygdalina prevents breast cancer by concurrently controlling several molecular targets related to mitosis, DNA synthesis, cellular metabolism, and oncogenic signaling. Such a systems-level mechanism demonstrates *V. amygdalina's* promise as a source of novel anticancer lead chemicals and is congruent with the therapeutic philosophy of many medicinal plants. The findings are based solely on computational predictions and require experimental validation through in vitro and in vivo studies.

5. CONCLUSION

Through a multi-target pharmacological mechanism including AURKA, TYMS, and numerous cancer-associated signaling pathways, including PI3K-Akt, MAPK, and Ras signaling, this study concludes that Vernonia amygdalina has strong anti-breast cancer potential. A solid theoretical basis for the future development of drugs derived from V. *amygdalina* as promising therapeutic candidates for the treatment of breast cancer is provided by the combined network pharmacology and molecular docking investigations. However, additional in vitro, in vivo, and clinical studies are needed to confirm the biological activities, molecular processes, safety, and therapeutic efficacy of these phytochemicals, as the current study is mostly based on computational predictions. All things considered, this work offers valuable insights into the molecular pharmacology of *Vernonia amygdalina* and lays a solid basis for its future advancement in precision oncology and breast cancer medication discovery.

DECLARATIONS

Acknowledgments

The authors wish to express sincere gratitude to all individuals and institutions whose support contributed to the successful completion of this study. The authors sincerely acknowledge the developers and maintainers of all bioinformatics platforms utilized in this study, including ADMETlab 3.0, SwissTargetPrediction, STRING, GEPIA2, PubChem, and the RCSB Protein Data Bank.

Conflicts of Interest

The authors declare no conflicts of interest

Contribution of Authors

T.A.S.: Conceptualization, methodology, data curation, formal analysis, visualization, writing – original draft, and writing – review & editing. A.S.: Supervision, validation, and writing – review & editing. A.S.F.: Methodology, data curation, and writing – review & editing. M.E.E.: Formal analysis, visualization, and writing – review & editing. M.I.O.: Investigation, resources, and writing – review & editing. All authors have read and approved the final version of the manuscript for submission.

Ethical approval

This study did not involve human participants, animals, or clinical samples; therefore, ethical approval was not required.

AI Statement

No generative artificial intelligence tools were used in the preparation of this manuscript.

6. REFERENCES

[1] World Health Orgnization . (2024). Breast cancer. https://www.who.int/news-room/fact-sheets/detail/breast-cancer

[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. (2021) 71:209–49. doi: 10.3322/caac.21660, PMID: [DOI] [PubMed] [Google Scholar]

[3] Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol. (2022) 95:20211033. doi: 10.1259/bjr.20211033, PMID: [DOI] [PMC free article] [PubMed] [Google Scholar]

[4] Gersten O, Wilmoth JR. The cancer transition in Japan since 1951. Demogr Res. (2002) 7:271–306. doi: 10.4054/DemRes.2002.7.5 [DOI] [Google Scholar]

[5] Abdel RO. The epidemiologic transition: a theory of the epidemiology of population change. Bulletin of the World Health Organization 79.2 (2021): 161–170. [PMC free article] [PubMed]

[6] Zhao J, Xu L, Sun J, Song M, Wang L, Yuan S, et al. Global trends in incidence, death, burden and risk factors of early-onset cancer from 1990 to 2019. BMJ Oncol. (2023) 2:e000049. doi: 10.1136/bmjonc-2023-000049, PMID: [DOI] [PMC free article] [PubMed] [Google Scholar]

[7] Kaur C, Kumar S, Singh S, et al. Cancer: a black spot to human race! Res J Sci Technol. 2020;12(1):1–12. [Google Scholar]

[8] Usunobun, U., & Ngozi, O. (2016). Phytochemical analysis and proximate composition of Vernonia amygdalina. *International Journal of Scientific World*, *4*(1), 11-14. https://doi.org/10.14419/ijsw.v4i1.5845

[9] Chandran U, Mehendale N, Patil S, et al. Network pharmacology. Innov Approaches Drug Discov 2017:127-164.

[10] Noor F, ul Qamar M, Ashfaq UA, et al. Network pharmacology approach for medicinal plants: Review and assessment. Pharmaceuticals 2022;15(5):572.

[11] Yang M, Chen JL, Xu LW, et al. Navigating traditional Chinese medicine network pharmacology and computational tools. Evid Based Complement Alternat Med 2013;2013:731969.

[12] Li L, Yang L, Yang L, et al. Network pharmacology: A bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. Chin Med 2023;18(1):146.

[13] Tallei TE, Fatimawali, Adam AA, et al. Molecular insights into the anti-inflammatory activity of fermented pineapple juice using multimodal computational studies. Arch Pharm (Weinheim) 2024;357(1):e2300422.

[14] Pendong CHA, Suoth EJ, Fatimawali F, et al. Network pharmacology approach to understanding the antidiabetic effects of pineapple peel hexane extract. Malacca Pharm 2024;2(1):24-32.

[15] Xiong, Z., Lo, H.P., McMahon, K.A., Martel, N., Jones, A., Hill, M.M., Parton, R.G., Hall, T.E. (2021) In vivo proteomic mapping through GFP-directed proximity-dependent biotin labelling in zebrafish. eLIFE.

[16] Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2023). PubChem 2023 update: improved data content and web interfaces. *Nucleic Acids Research, 51*(D1), D1373–D1380. https://doi.org/10.1093/nar/gkac956

[17] Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research, 47*(W1), W357–W364. https://doi.org/10.1093/nar/gkz382
[18] Safran, M., Rosen, N., Twik, M., et al. (2021). The GeneCards Suite. In *Practical Guide to Life Science Databases*. Springer. https://doi.org/10.1007/978-981-16-5812-9_21
[19] Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., ... & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research*, *13*(11), 2498-2504.https://doi.org/10.1101/gr.1239303 PMID: 14597658
[20] Oliveros, J. C. (2015). Venny 2.1: An interactive tool for comparing lists with Venn diagrams. Retrieved from https://bioinfogp.cnb.csic.es/tools/venny/
[21] Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., ... & Von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic acids research*, *51*(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000
[22] Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics, 36*(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931
[23] Janeček, M., Rossmann, M., Sharma, P. *et al.* Allosteric modulation of AURKA kinase activity by a small-molecule inhibitor of its protein-protein interaction with TPX2. *Sci Rep* **6**, 28528 (2016). https://doi.org/10.1038/srep28528
[24] Czyzyk DJ, Valhondo M, Jorgensen WL, Anderson KS. Understanding the structural basis of species selective, stereospecific inhibition for Cryptosporidium and human thymidylate synthase. FEBS Lett. 2019 Aug;593(15):2069-2078. doi: 10.1002/1873-3468.13474. Epub 2019 Jun 18. PMID: 31172516; PMCID: PMC6690774.
[25] Burley, S. K., Bhikadiya, C., Bi, C., Bittrich, S., Chen, L., Crichlow, G. V.,. & Zhuravleva, M. (2021). RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. *Nucleic acids research*, *49*(D1), D437-D451. https://doi.org/10.1093/nar/gkaa1038
[26] Fu, L., Shi, S., Wu, C., Zeng, X., Cao, D., & Hou, T. (2024). ADMETlab 3.0: An updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. *Nucleic Acids Research, 52*(W1), W422–W431. https://doi.org/10.1093/nar/gkae236
[27] Li Y, Lu D, Xu A. Abstract TP276: Identification of Potential Key Genes and Pathways in Ischemic Stroke by Analysis of Human High Throughput Sequencing Data. Stroke 2020;51(1). ATP276-ATP276.
[28] Wang H, Li Y, Liu Y. ADMET and drug-likeness prediction of natural compounds with anti-inflammatory potential: An in silico study. J Biomolecul Struct Dyn 2021; 39(18):6827–39. https://doi.org/10.1080/07391102.2020.1806898.
[29] Oncology Hanahan D, Weinberg RA. *Hallmarks of cancer: the next generation.* Cell. 2011;144(5):646–674.
[30] Molecular Biology Fu L, Kettner NM. *The circadian clock in cancer development and therapy.* Progress in Molecular Biology and Translational Science. 2013;119:221–282.
[31] Cancer Research Barker N, Clevers H. *Tracking down the stem cells of the intestine: strategies to identify adult stem cells.* Gastroenterology. 2007;133(6):1755–1760.
[32] Molecular Biology Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. *The protein kinase complement of the human genome.* Science. 2002;298(5600):1912–1934.
[33] Molecular Oncology Hanker AB, Sudhan DR, Arteaga CL. *Overcoming endocrine resistance in breast cancer.* Cancer Cell. 2020;37(4):496–513.
[34] Saddala MS, Huang H. Identification of novel inhibitors of tumor necrosis factor alpha using combined ligand-based and structure-based virtual screening approaches. J Molecul Graph Modell 2019;92:238–49. https://doi.org/10.1016/j. Jmgm.2019.07.008.
[35] Kim JY, Park SJ, Yun KJ. Alpha- and beta-amyrin inhibit inflammatory responses mediated by TLR4/NF-κB signaling pathway in macrophages. Planta Med 2016;82 (7):603–9. https://doi.org/10.1055/s-0042-101925

[36] Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*. 2019;47(W1):W357–W364.
[37] Xiong G, Wu Z, Yi J, et al. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Research*. 2021;49(W1):W5–W14.
[38] Nguyen NH, Nguyen MT, Little PJ, et al. Vernolide-A and Vernodaline: sesquiterpene lactones with cytotoxicity against cancer. *Journal of Environmental Pathology, Toxicology and Oncology*. 2020;39(4):299–308.
[39] Luo Y, Zhang D, Hou L, Lin N. Vernodalin suppresses tumor proliferation and increases apoptosis of gastric cancer cells through attenuation of FAK/PI3K/AKT/mTOR and MAPKs signaling pathways. *Current Pharmaceutical Biotechnology*. 2023;24(5):708–717.
[40] Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nature Chemical Biology*. 2008;4(11):682–690.
[41] Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chinese Journal of Natural Medicines*. 2013;11(2):110–120.
[42] Nikonova AS, Astsaturov I, Serebriiskii IG, Dunbrack RL Jr, Golemis EA. Aurora A kinase (AURKA) in normal and pathological cell growth. *Cellular and Molecular Life Sciences*. 2013;70:661–687.
[43] Du R, Huang C, Liu K, Li X, Dong Z. Targeting AURKA in cancer: molecular mechanisms and opportunities for cancer therapy. *Molecular Cancer*. 2021;20:15.
[44] Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer*. 2003;3(5):330–338.
[45] Fu L, Kettner NM. The circadian clock in cancer development and therapy. *Progress in Molecular Biology and Translational Science*. 2013;119:221–282.
[46] Compton DA. Mechanisms of aneuploidy. *Current Opinion in Cell Biology*. 2011;23(1):109–113.
[47] Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science*. 2002;298(5600):1912–1934.
[48] Hanker AB, Sudhan DR, Arteaga CL. Overcoming endocrine resistance in breast cancer. *Cancer Cell*. 2020;37(4):496–513.