RESEARCH ARTICLE

Editorial Process: Submission:08/26/2025 Acceptance:04/06/2026 Published:04/07/2026

Molecular Insights into Identification of Natural AKT1/mTOR Signaling Inhibitors from *Veratrum Viride*-Derived Alkaloids for Breast Cancer Treatment: A Comprehensive Analysis Using Network Pharmacology, Molecular Docking, and Molecular Dynamics

Anu Priya Eswaran, Selvaraj Jayaraman, Sathanraj Raj Natarajan, Vishnu Priya Veeraraghavan*

Abstract

Objective: Breast cancer (BC) is a complex illness that affects millions of women globally. As its incidence rises, new treatment strategies are needed. *Veratrum viride*, a traditional medicinal herb, is known for its therapeutic potential, yet its molecular mechanism of action against BC remains unclear. The purpose of this preliminary investigation is to assess *V. viride*'s anti-breast cancer potential by identifying its active compounds and using bioinformatics techniques to clarify their multi-target mechanisms. **Materials & methods:** Initially, eleven compounds from *V. viride* were examined for pharmacokinetic and toxicity characteristics. Network pharmacology was used to predict and integrate compound–target interactions with genes linked to BC. Topological and drug–protein interaction (DPI) analyses were employed to identify important hub genes. KEGG pathway enrichment and Gene Ontology (GO) analyses were conducted to validate functional relevance. Target–compound interactions were verified through molecular docking and molecular dynamics simulation analysis. **Results:** Using ADMET profiling analysis and drug-likeness properties, three alkaloids namely jervine, veratramine, and rubijervine were identified as promising drug candidates. We identified six important hub genes: *MTOR, INSR, FOXO1, FOXO3, RPS6KB1*, and *AKT1*. According to GO and KEGG analyses, the compounds targeted pathways important in the regulation of BC, including AMPK, HIF-1, FOXO, and PI3K/AKT/mTOR. Moreover, jervine demonstrated robust binding stability and affinity with core targets in molecular docking and dynamics simulations. **Conclusion:** This work provides the first evidence that alkaloids derived from *V. viride*, especially jervine, may act as multi-target inhibitors against BC. However, further experimental validation is required to confirm their therapeutic efficacy.

Keywords: Bioactive compounds- BC targets- Hub genes- Molecular docking- Multitargeting- Signaling pathways

Asian Pac J Cancer Prev, **27 (4)**, 1335-1350

Introduction

Breast cancer (BC) is a diverse malignancy originating in the mammary glands [1]. With 2.3 million cases, it accounts for 11.7% of all cancers, making it most recurrent cancer in women globally. In most nations, it is the most often diagnosed cancer; one in four cancer cases and one in six cancer-related deaths [2]. Since BC is usually diagnosed at an earlier stage with limited therapeutic options, patients with breast cancer typically have a bad prognosis [3]. BC can be treated with surgery, chemotherapeutic agents, immunotherapy, and focused treatments nowadays. Since the use of many chemotherapy drugs elevates side effects it also exacerbates resistance to tumors which severely affects the survival rate of patients and thereby complicates BC treatment .Thus, there is a demand in search for alternative treatment to create safe and efficient anticancer treatments to assist to enhance BC therapy [4]. This underlines the need of looking at bioactive molecules taken from medicinal plants as appealing, cost-effective substitutes with less side effects for cancer treatment. Medicinal plants contain diverse bioactive compounds which are valuable for treating different diseases with therapeutic possibilities [5, 6].

*Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600077, India. *For Correspondence: vishnupriya@saveetha.com*

Moreover, wide range of studies have confirmed that bioactive compounds like polyphenols, alkaloids, terpenes, and polysaccharides obtained from medicinal herbs exhibits remarkable anti-oxidant, anti-cancer, anti-inflammatory, cardioprotective, hepatoprotective, immunomodulatory, neuroprotective, and anti-diabetic properties [6]. In this regard, modern bioinformatics techniques can be utilized which may help to understand the complicated pharmacological features of medicinal herbs.

In this study, the *Veratrum* spp. has been chosen which was mostly derive from roots and rhizomes and found to have high steroidal alkaloid content. Among other things, these alkaloids have bradycardic, analgesic, and anti-cancer actions [7, 8]. Moreover, some of them including *V.californicum, V. viride, V. album*, and *V. nigrum* investigated Even though around sixteen species of Veratrum found to have bioactive compounds particularly, despite extensive research on many Veratrum steroidal alkaloids particularly some medicinal properties of *V.viride* remain unexplored, with limited spectral data available. Hence, as a result, *Veratrum viride* has been chosen for this study [7]. *Veratrum viride* is traditionally used to treat hypertension, rheumatism [8–11] and majorly phytochemicals particularly steroidal alkaloids present in it found to be in use as a conventional medicine for rheumatism and analgesic disorders [7, 8]. However, although traditional uses and some pharmacological effects are documented, the specific biological mechanism mentioning especially how *Veratrum viride* and its steroidal alkaloids exert anticancer effects, particularly against breast cancer (BC) remains unclear.

This gap highlights the necessity for thorough research into the anticancer potential of *Veratrum viride* and its active constituents in the context of BC, particularly focusing on clarifying the potentially intricate molecular mechanisms that contribute to its antitumor effects using in silico analysis. The flowchart of this study is presented in (Figure 1).

Materials and Methods

Selection of Bioactive compounds

From the article review, the plant species *Veratrum viride* was identified as relevant one. The extract of this plant has been determined to contain potential medicinal properties against numerous diseases and cancer [7, 12, 13]. In this study, utilizing Indian Medicinal Plant, Phytochemical bioactive compounds And Therapeutics (IMPAAT) data repository (https://cb.imsc.res.in/imppat/) [14], the secondary metabolites of the identified plant species was acquired and once after retrieving the compounds, duplicates were removed.

Drug-Likeness and Pharmacokinetics (ADME) Screening

The drug alikeness and pharmacokinetic property of every retrieved bioactive compounds namely Jervine,Veratrosine, Neogermitrine,Veratramine,Rubijervine, Proveratrine-A, Isorubijervine, Germerine, Germitrine, Germidine and Proveratrine-B was identiifed through deriving the Simplified Molecular Input Line Entry System (SMILES) from the PubChem data repository (https://pubchem.ncbi.nlm.nih.gov/). Further, Lipinski's Rule of Five which forecasts oral bioavailability, was analysed to evaluate drug-likeness for these compounds. The inclusion criteria includes < 500 kDa and a count of < 5 hydrogen (H) bond donors (NHBD) and < 10 H bond receptors (NHBA) and a Log P value of not more than 5. Further, the SMILES of the compounds were been subjected to Swiss ADME online web server (http: //www. swissadme.ch/) [15] then the drug likeness, pharmacokinetics was predicted. Notably, the pharmacokinetics property comprises of factors including absorption, distribution, metabolism and excretion

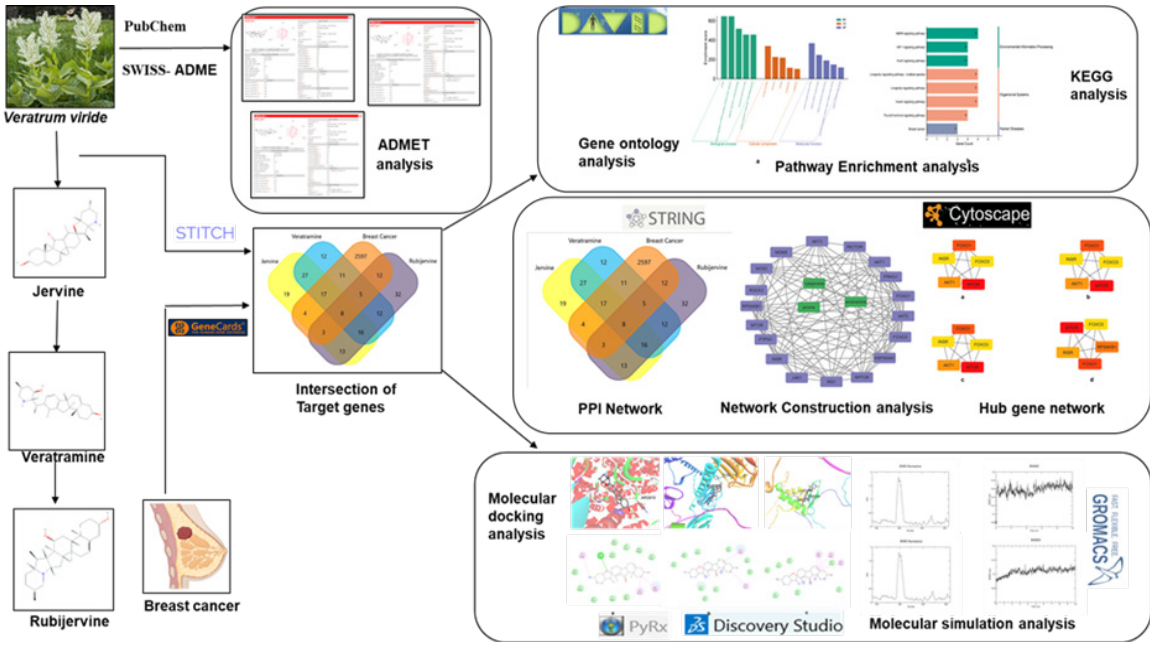


Figure 1. The Workflow of *Veratrum viride* against BC via Network Pharmacology and Molecular Docking and Simulation Analysis

(ADME) was identified for every compound, which determines their suitability as possible drug. Furthermore, the several physicochemical characteristics including polar surface area (TPSA), topological characteristics, and molar refractivity was also identified.

Target prediction

Target prediction analysis for the prime bioactive compounds was carried out using Swiss Target Prediction (http://www.swisstargetpredurden.ch/) [16] database which demonstrates the mechanism of action and identifies the possible targets for them. In this study, the database was accessed with selection to species as "Homo sapiens" with probability ratio greater than 0 and then the efficient target genes of steroidal compounds were identified.

Identification of BC related target dataset

GeneCards database (https://www.genecards.org/) [17], a complete platform combining gene-related data from several sources, which offers well-chosen information on molecular interactions, pathways, gene functions, and linked diseases. The gene targets linked to breast cancer (BC) was curated using the keyword "Breast Cancer". This method helps one to grasp important genetic players involved in the development of diseases and their possible interactions with bioactive compounds.

Common targets of Veratrum viride in Treating BC

An analysis of the compound-disease targets was carried out to analyse common targets between BC and compounds. In this study, using Funrich tool (http://www.funrich.org) [18] bioactive compounds' targets and comparison with established breast cancer (BC) targets were performed. The resulted Venn diagram presented genes that overlapped, indicating possible BC treatment targets.

Drug–Protein Interaction (DPI) network construction and hub gene analysis

The overlapped genes were exposed to next analysis utilising the STITCH (Search Tool for Interacting Chemicals) (data repository [19]. STITCH database (http://stitch.embl.de/) was used and drug- protein interaction network was constructed by selecting "Homo sapiens" as specific gene of interest with a combined score of >0.08 as standard one implicating proteins reliability. Followed by this, cytoscape software (version 3.5.1; http://www.cytoscape.org) was adopted to visualize the resultant DEGs network, within which cytohubba plugin was utilised and hub genes were detected. Based on node ranking, topological analysis (radiality, degree, proximity, and MCC) identified hub genes, and a Venn diagram was employed to determine four typical targets. Depending on the total score, the DPI network's edge widths indicated the strength of the interactions [20].

Prognostic validation of Veratrum viride associated BC targets and analysis of its stage status

GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepia.cancer-pku.cn/) was subjected to evaluate the survival rates and identify the correlating crucial targets in BC patients [21]. Further, survival analysis was carried out which accompanied by log-rank tests by Kaplan Meier plots. The mRNA expression levels of potent targets compared with early and advanced stages were examined using TCGA-BRCA datasets from GEPIA 2.0 database. Additionally, Statistical significance ($p < 0.05$) was used to assess gene expression–survival associations, while hub gene expression and prognostic relevance across BC stages and metastasis were evaluated via GEPIA

Pathway enrichment and functional profiling

DAVID (Database for Annotation, Visualization, and Integrated Discovery) database (https://david.ncifcrf.gov/) a strong tool for performing functional enrichment analysis was adopted to identify hub gene targets related to BC treatment. This analysis involves combination of identifying target related biological processess and signalling pathways, this includes Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses [22]. The hub gene role in BC-related pathways were identified using KEGG analysis, whereas GO enrichment analysis evaluated the hub genes' cellular components (CC), molecular functions (MF), and biological processes (BP). Later, SRplot was utilised to display the web interconnection of active components crucial targets, compounds and molecular pathways.

Molecular docking analysis

Molecular docking, which provides information on multitarget activity and possible synergistic drug target interactions, was used to investigate the link between key targets and core bioactive molecules and validate network pharmacology predictions [23]. Adopting the PubChem repository (https://pubchem.ncbi.nlm.nih.gov/), 3D dimensional patterns of jervine, veratramine, rubijervine along with standard drug Tamoxifen for comparison was retrieved and using RCSB PDB (https://www.rcsb.org/) database by selecting "Homo sapiens" as source organism, 2.5A as refinement resolution, the 3D structure of the target proteins namely MTOR, INSR, AKT1 and RPS6KB1 encoded by the hub gene was isolated as a complete protein sequence with ligand models. Docking were conducted using the Lamarckian genetic algorithm (LGA) with nearly upto 100 cycles in PyRx [24]. Further, using Discovery Studio Visualizer software 3D and 2D docked results were then visualized and analyzed [23].

Molecular Dynamics (MD) analysis

In order to simulate protein-ligand interactions using molecular dynamics (MD), the GROMACS (GROningen MAchine for Chemical Simulations package) [25] was utilised. Further, PyMOL [26] was utilized to separate the docked protein-ligand complex into its individual protein and ligand components for the MD simulation. The protein topology was constructed in GROMACS adopting the Amber99sb-ildn force field, when the ligand topology was created with the ACPYPE package [27]. These topologies were then joined to form the complete protein-ligand complex topology, enabling the MD simulation

Table 1. Isolated Bioactive Compounds from *Veratrum viride*

Indian medicinal plant	IMPPAT phytochemical identifier	Phytochemical name	Reference ID
Veratrum viride	IMPHY000366	Jervine	ISBN:9788172362140
Veratrum viride	IMPHY005194	Veratrosine	ISBN:9788172362140
Veratrum viride	IMPHY005675	Neogermitrine	ISBN:9788172362140
Veratrum viride	IMPHY006313	Veratramine	ISBN:9788172362140
Veratrum viride	IMPHY006541	Rubijervine	ISBN:9788172362140
Veratrum viride	IMPHY007297	Protoveratrine A	ISBN:9788172362140
Veratrum viride	IMPHY007333	Iso rubijervine	ISBN:9788172362140
Veratrum viride	IMPHY008715	Germerine	ISBN:9788172362140
Veratrum viride	IMPHY008716	Germitrine	ISBN:9788172362140
Veratrum viride	IMPHY009634	Germidine	ISBN:9788172362140
Veratrum viride	IMPHY009798	Protoveratrine B	ISBN:9788172362140

to proceed. A dodecahedron box (1.0 nm) with periodic boundary conditions was used for the prepared complex. The TIP3P water model and NaCl ions were added for solvation and system neutralization. Energy minimization was performed for 1000 steps, followed by equilibration using MDP files from the Lekumal protein-ligand MD simulation tutorial [28] with 300 ps of equilibration at 300K under NVT conditions and 500 ps under NPT at 1 bar pressure. Based upon equilibration, a 10 ns molecular dynamics simulation was carried out and the trajectory data were recorded in XTC format.

Ethical statement

The present investigation exclusively employed publicly available bioinformatics databases and analytical instruments; therefore, ethical clearance for human or animal participants was not required.

Helinski rule

All bioinformatics analyses were performed in compliance with the statement of Helsinki's tenets, guaranteeing that ethical issues were suitably acknowledged and maintained during the entire research procedure.

Statistical analysis

The data verification for this study was performed using the statistical computer language R, which is used for statistical computing. P-values for the analysis of mRNA expressions and the development of Kaplan-Meier plots to evaluate overall survival were calculated using the log-rank test.

Results

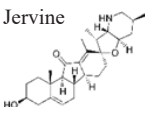
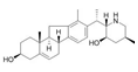
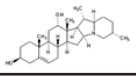
Bioactive compounds screening

A total of about 11 bioactive compounds namely Jervine, Veratrosine, Neogermitrine, Veratramine, Rubijervine, ProtoveratrineA, Isorubijervine, Germerine, Germitrine, Germidine and Protoveratrine B were retrieved from the plant *Veratrum viride* searching the IMPPAT database (Table 1).

Drug likeness and ADME/T profiling

Using SwissADME tool, drug-likeness and pharmacokinetic analyses were performed on all 11 isolated compounds. Compounds that followed drug alikeness properties encompassing Lipinski's rule of five, good oral bioavailability, and those with a TPSA score less than 90 were deemed as possible drug candidates for drug-likeness assessment. Further, compounds with high gastrointestinal (GI) absorption, favorable P-glycoprotein (P-gp) substrate characteristics and interactions with isoenzymes of cytochrome P450 (CYP) (Table 2, 3) were considered potential for pharmacokinetic analysis. Notably, three compounds namely Jervine, Veratramine, and Rubijervine was found to emerged

Table 2. Drug Likeness of Bioactive Compounds

S.no	Molecule	Molecular formula	Mol. Wt.	TPSA	ESOL Class	Lipinski's violation	Bioavailability Score	NHBA	NHBD	Log-P
1	Jervine 	C27H39NO3	425.6	58.56	Moderately soluble	0	0.55	4	2	3.89
2	Veratramine 	C27H39NO2	409.6	52.49	Moderately soluble	1	0.55	3	3	4.12
3	Rubijervine 	C27H43NO2	413.64	43.7	Moderately soluble	1	0.55	3	2	4.3

NHBD, Number of Hydrogen Bond Donors; NHBA, Number of Hydrogen Bond Acceptors

Table 3. Pharmacokinetics of Bioactive Compounds

S.No	Compounds	GI absorption	P-gp substrate	CYP1A2 inhibitor	CYP2C 19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	Jervine	High	Yes	No	No	No	No	No
2	Veratramine	High	Yes	No	No	No	No	No
3	Rubijervine	High	Yes	No	No	No	No	No

as promising candidates and may represent the key bioactive constituents underlying the anti-breast cancer (anti-BC) potential of *Veratrum viride*.In contrast, the eight compounds namely Veratrosine, Neogermitrine, Protoveratrine-A, Isorubijervine, Germerine, Germitrine, Germidine, and Protoveratrine B were excluded from further consideration due elevated TPSA values, low oral bioavailability , poor GI absorption, absence of P-gp substrate behavior and inhibitory actions with isoenzymes of CYP. Accordingly, these eight compounds were not subjected to further analyses in the present study.

Target prediction for bioactive compounds and BC

Based on previous analysis, the target prediction analysis was performed on three compounds namely Jervine, Veratramine, and Rubijervine which demonstrated no toxicity and were identified as promising candidates. Further, SMILES (canonical) for each one of the compound were collected from the PubChem database and submitted into the Swiss Target prediction tool. Only target genes/specific genes were predicted to interact with each compound within a probability score of 0.1 or higher. In total, the three compounds were predicted to collectively interact with approximately 300 potential genes, indicating a wide range of biological activity and therapeutic effects. After removing duplicates, 106 unique target genes were identified (Supplementary Table 1-3). Further, from the Gene cards database overall of 2,657 unique genes related to BC was identified (Supplementary Table 4).

Bioactive compound and disease target interaction network

A Venn diagram was built to screen the overlapping genes among the 3 compounds and the specific genes association with it. Jervine, Veratramine, and Rubijervine (300 genes) and the genes associated with BC (2657 genes). Further, analysis showed eight (8) overlapping genes, highlighting the similar targets between BC and the compounds datasets. These shared genes demonstrates a possible therapeutic connection, which emphasizes the need of more research on the possible function of these molecules and warranting further investigation into their therapeutic potential of breast cancer (BC).The results given in (Figure 2(a)), illustrates convergence between compound and BC targets through Venn diagram.

Identification of DPI network and Hub genes

The STITCH database was used to further investigate the collection of eight genes including *AKT2, AKT1, AKT3, JAK1, PRKG1, ROCK2, RPS6KB1*, and *INSR* obtained via the Venn diagram analysis and its relationship to the substances jervine, veratramine, and rubijervine targets. (Figure 2(b)) shows the compound target network of jervine, veratramine, and rubijervine with their potential targets, constructed using STITCH.Cytoscape software version 3.5.1 was used to visualize the network, which revealed 21 nodes and 106 edges, depicted in

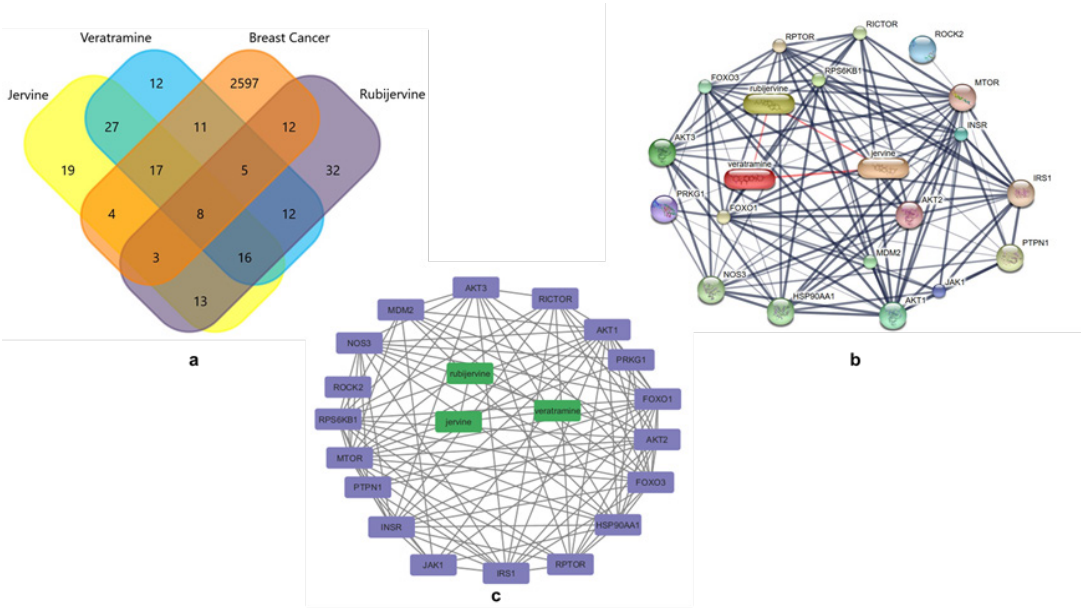


Figure 2. a. A Venn diagram presenting the overlapping between compound targets and BC; b. Compound target network; c. Drug-protein interaction network

Table 4. Network Profiling through Cytoscape Integrated with the CytoHubba Plugin

Gene Name	Degree	Closeness	Radiality	MCC
MTOR	17	17	1.865	587041
INSR	14	15.5	1.714	580440
RPS6KB1	14	15.5	1.714	585360
AKT1	15	16	1.764	545760

(Figure 2(c)). The top five nodes among the eight genes were identified using various ranking criteria, including MCC, Radiality, Closeness, and Degree. Hub genes *MTOR, INSR, FOXO3, FOXO1, AKT1*, and *RPS6KB1* were found. These hub genes may be engaged in the anti-cancer action of the compounds under study because they important roles in the network of drug-protein interactions. Based on topological scores, (Figure 3 (a-d)) shows the interaction between compound targets and its top five engaging connective hub genes that the compounds target, and (Figure 3 (e)) shows the four common targets found among the five hub genes listed in (Table 4).

Prognostic validation of target genes from bioinformatic analysis

Tumorigenesis is a process that disrupts key signaling pathways such as PI3K/AKT/mTOR, Wnt/β-catenin, and MAPK, leading to uncontrolled cell proliferation and metastasis. Evaluating gene expression and survival analyses can help identify deregulated genes involved in the tumorigenic process [29]. In this study,the potential significance of selected target genes (MTOR, INSR, RPS6KB1, and AKT1) associated with the compounds jervine, veratramine, and rubijervine in breast cancer (BC), was examined through extensive mRNA expression and survival plot analyses employing the GEPIA database, in order to validate the previous findings. Notably, the mRNA expression levels in BC tissues, revealed a notable upregulation of the genes MTOR, RPS6KB1, AKT1, and INSR, as presented in (Figure 4(a)).These findings demonstrates the deregulation of these genes in BC, by further supporting their crucial relevance in the disease. Additionally, survival analysis was carried out using the GEPIA database, which showed upregulated levels of MTOR, RPS6KB1, AKT1, and INSR genes with poor survival outcomes as shown in (Figure 4(b)). Considering their values of hazard ratios (HR) and corresponding p-values. These four genes mTOR, RPS6KB1, AKT1, and INSR exhibited hazard ratios (HR) of 1, 1, 1.3, and 1.1 respectively, with corresponding p-values of 0.88, 0.83, 0.13, and 0.56. Furthermore, expression levels for AKT1, RPS6KB1, MTOR, and INSR in early and advanced stags were examined using GEPIA database. The stages expression levels were also suggested that advanced stages (Stage X) show higher level compared to lower level (stage I) as shown in (Figure 5). These observed values suggests that these genes may play role as potential prognostic markers supporting their involvement in the tumorigenesis of breast cancer even though not all results were statistically significant.

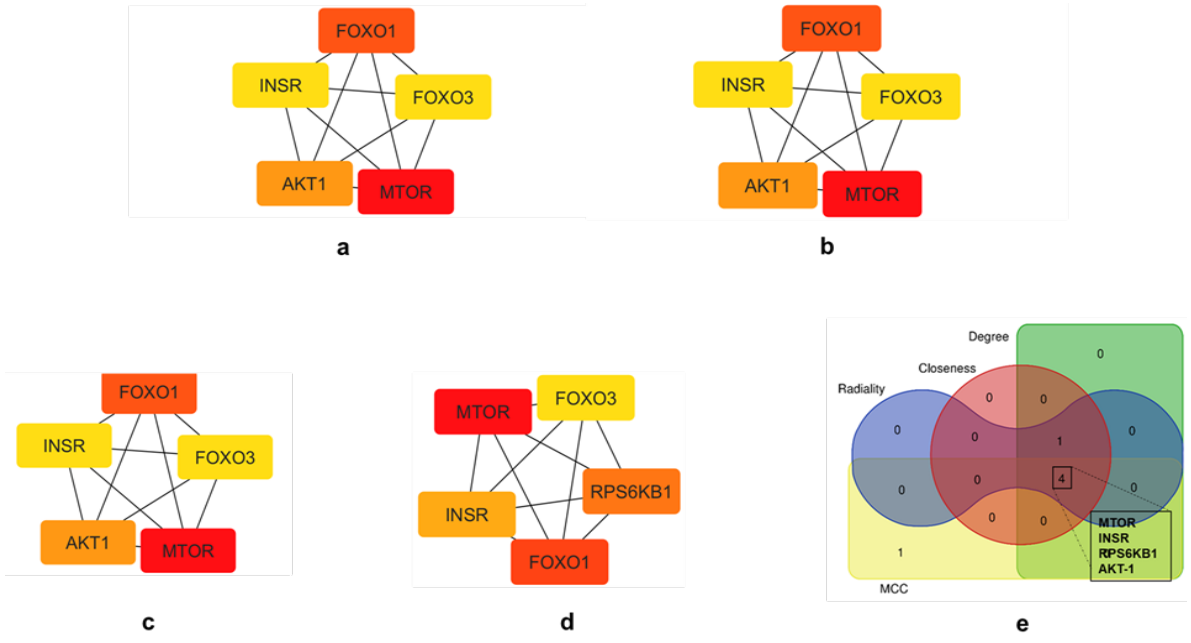


Figure 3. Top Five Core Targets Detected by a. Radiality, b. Closeness, c.Degree, d. MCC. Red color highest degree, yellow color lowest degree. e. Common four targets identified among five core targets using venn diagram. FOXO1: Forkhead box protein-1, FOXO3: Forkhead box protein 3, MTOR- Mammalian Target of Rapamycin, INSR- Insulin Receptor, AKT1- Serine Threonine Kinase-1, RPS6KB1- Ribosomal Protein S6 Kinase Beta-1.

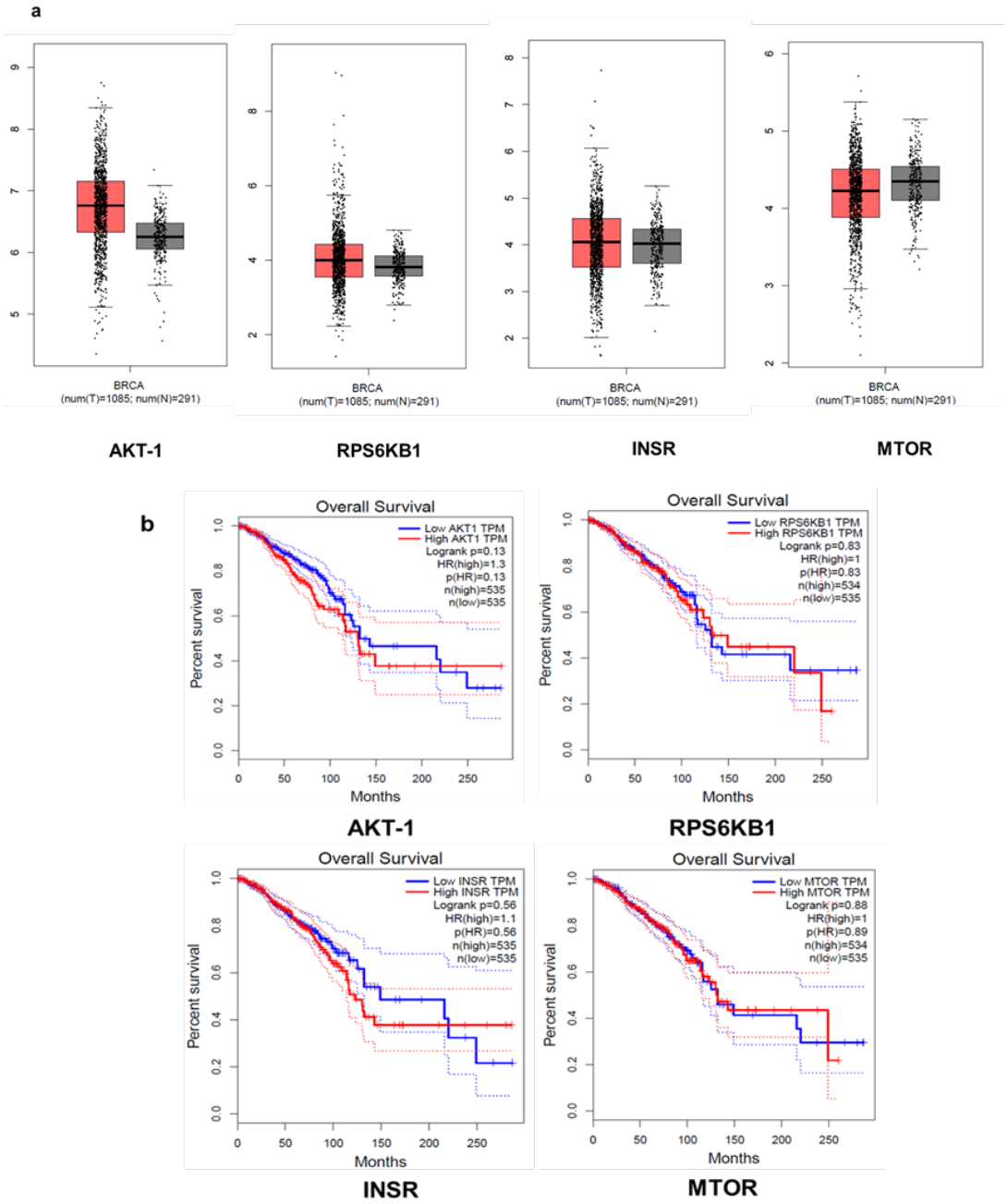


Figure 4. Prognostic validation of mRNA Expression and Overall Survival Analysis of Targets in Breast Cancer (BC). a. The GEPIA database was used to examine the target genes AKT1, RPS6KB1, INSR, and MTOR's mRNA expression levels. Plots from BC datasets show tumor tissue samples (n=1085) in grey and normal tissue samples (n=21) in orange. b. GEPIA also provided the overall survival curves for AKT1, RPS6KB1, INSR, and MTOR. Blue color denotes low expression levels and red color denotes high gene expression in the graphs. (MTOR: Mammalian Target of Rapamycin, RPS6KB1: Ribosomal Protein S6 Kinase B1, INSR: Insulin Receptor and AKT1: AKT Serine/Threonine Kinase 1).

Enrichment analysis

GO, KEGG enrichment, and functional annotation analyses was performed in order to find out *Veratrum viride*'s therapeutic action, where this analysis evaluates its mechanism of action and biological processes against BC treatment. DAVID database was utilised to identify the top five enriched GO terms for every category, ranked according to the count of functional annotations (Figure 6(a)). These targets were associated with key biological processes (BP), which includes anoikis, negative regulation of PERK-mediated unfolded protein response, suppression of macroautophagy, behavioral response to pain, and positive regulation of glycogen biosynthesis and Enriched cellular components(CC) which were mainly localized to the nuclear envelope, lysosome, dendrite, nucleoplasm, and membrane and Molecular functions(MF) which were predominantly related to protein kinase, hydrolase, protein tyrosine kinase, histone H2AS1 kinase activity, histone H2AXS139 kinase activity, eukaryotic translation initiation, Factor 2 alpha kinase activity and histone H3S28 kinase activities. Subsequently, KEGG pathway analysis (Figure 6(b))

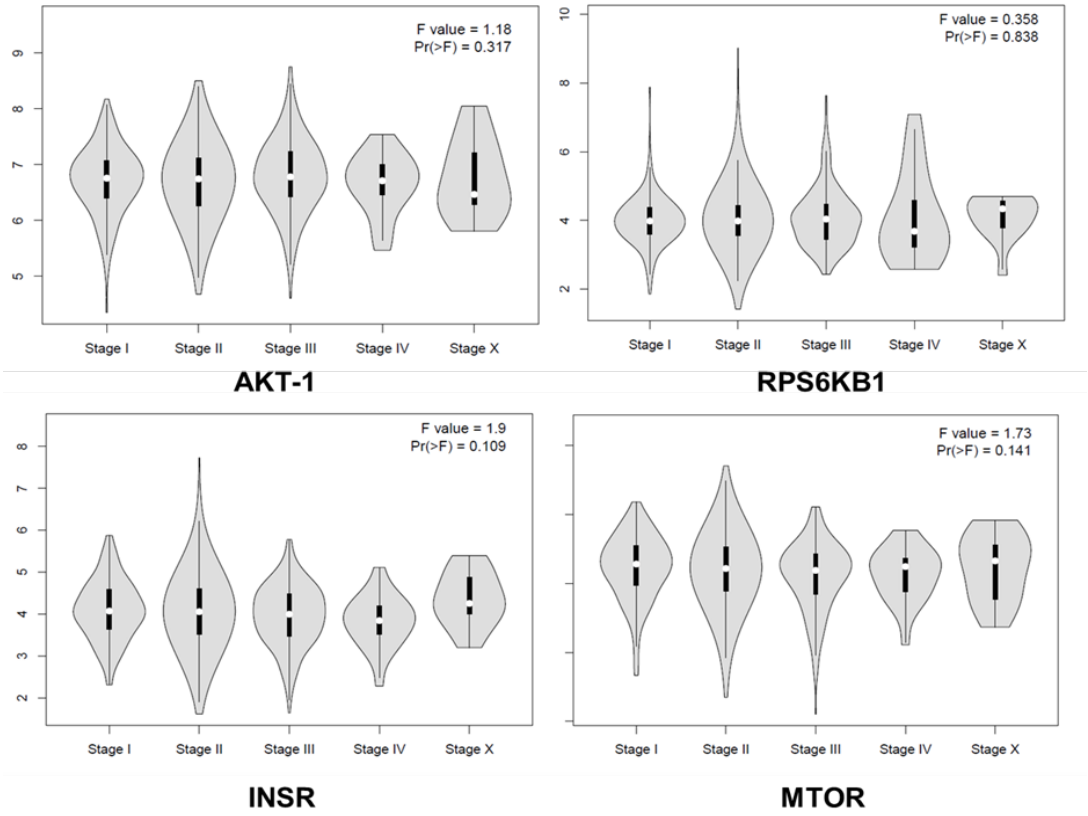


Figure 5. Expression Patterns of Key Genes in Breast Cancer were Analyzed by Retrieving mRNA Levels of *AKT1, RPS6KB1, INSR,* and *MTOR* Across Stages I to X from the GEPIA Database.

further revealed significant enrichment in AMPK, HIF-1, FOXO, insulin, thyroid hormone, and longevity-regulating pathways, along with breast cancer–associated pathways. Collectively, these findings enhance our knowledge on the functional roles of jervine, rubijervine, and veratramine and highlight their involvement in dysregulated pathways linked to breast cancer (BC).

Molecular docking and molecular dynamics (MD)

Furthermore, to determine close proximity interactions at molecular stage and to assess the molecular and binding mechanisms through which the compounds interact with the target proteins molecular docking analysis was performed. Based upon the degrees of common targets in the DPI network and hub targets collected by PPI interaction through cytoHubba and KEGG results, four common targets namely MTOR, INSR, AKT1 and RPS6KB1 were identified and the selected compounds Jervine, Veratramine, Rubijervine and tamoxifen were considered for the analysis. The outcome of our analysis

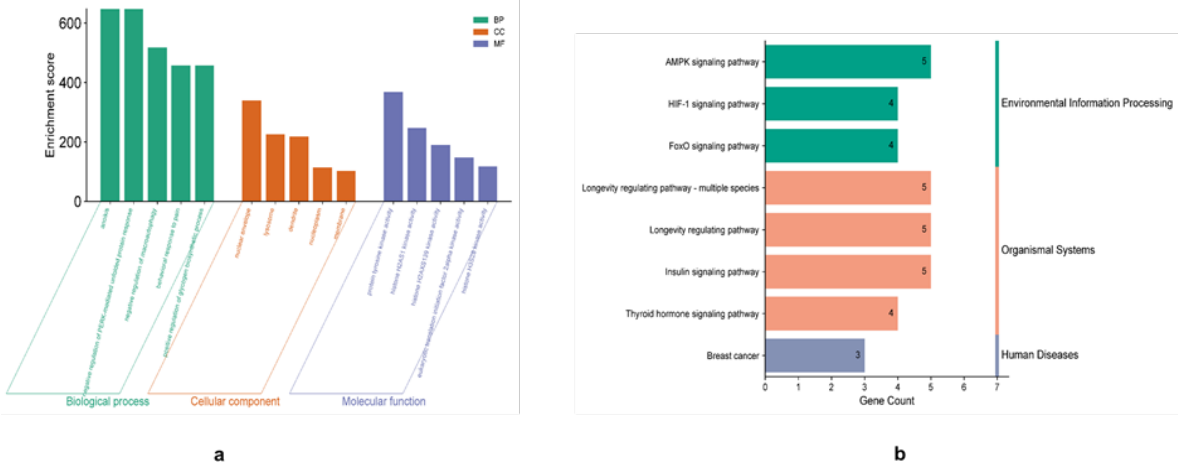


Figure 6. Key Targets' Enriched Pathways and Gene Ontologies from the BC Dataset. a. A bar chart was created using the gene ontologies (BP, CC, and MF). b. The highly enriched KEGG pathways were ranked and displayed based on gene counts across major categories, including environmental information processing, biological systems, and human diseases

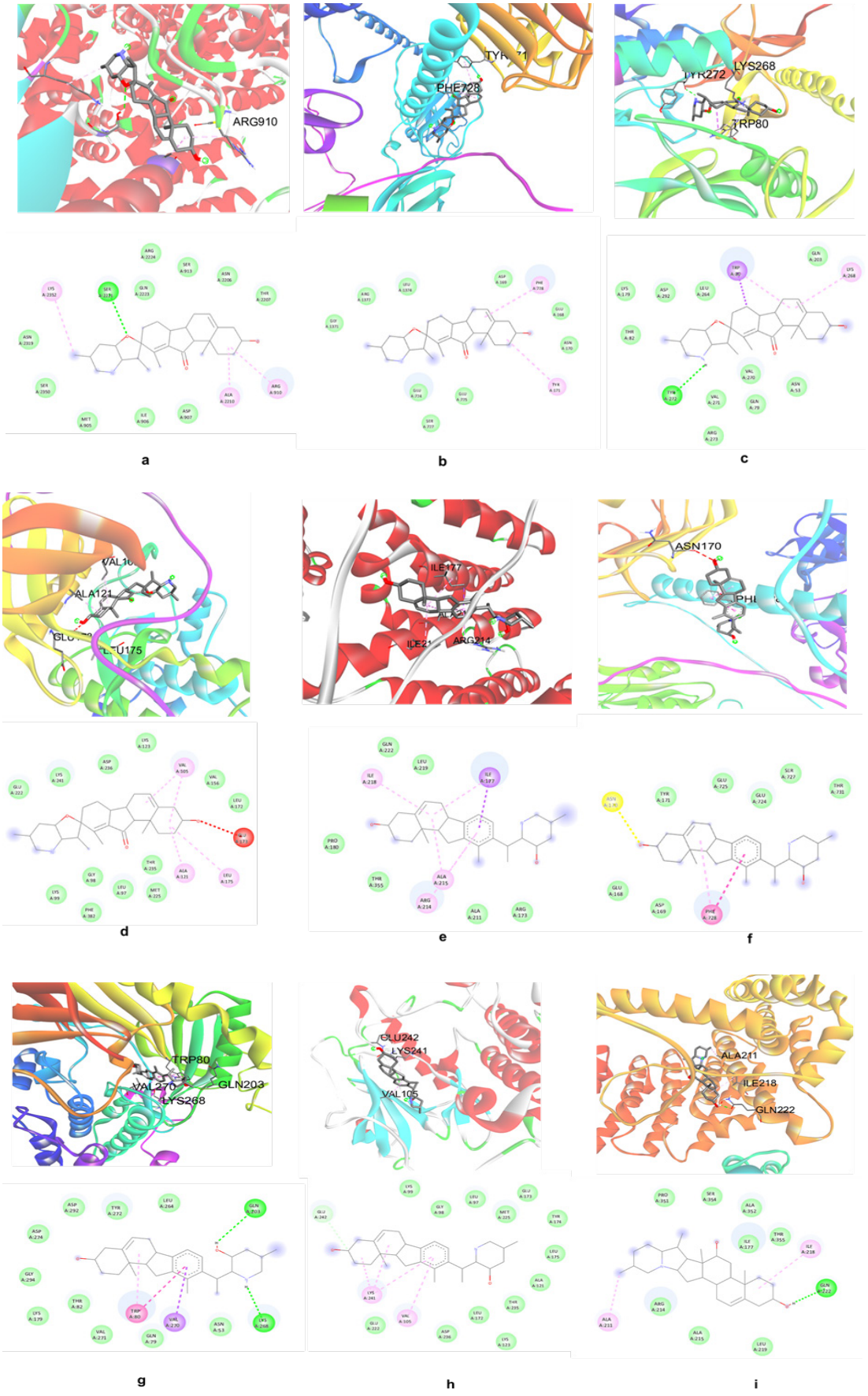


Figure 7. Binding Association of Jervine, Veratramine and Rubijervine with BC Key Targets by Molecular Docking. Analysis by PyRx software illustrates the binding complexes formed between jervine and the following proteins including MTOR, INSR, AKT-1 and RPS6KB1 (a-d), (e-h) represents the binding interactive complexes formed between veratramine and MTOR, INSR, AKT1 and RPS6KB1 proteins (i-l) depicts the interactive complexes formed between rubijervine and the proteins MTOR, INSR, AKT1 and RPS6KB1 targets. The 2D and 3D structural illustrations presented here were visualized using the Biovia Discovery Studio tool.

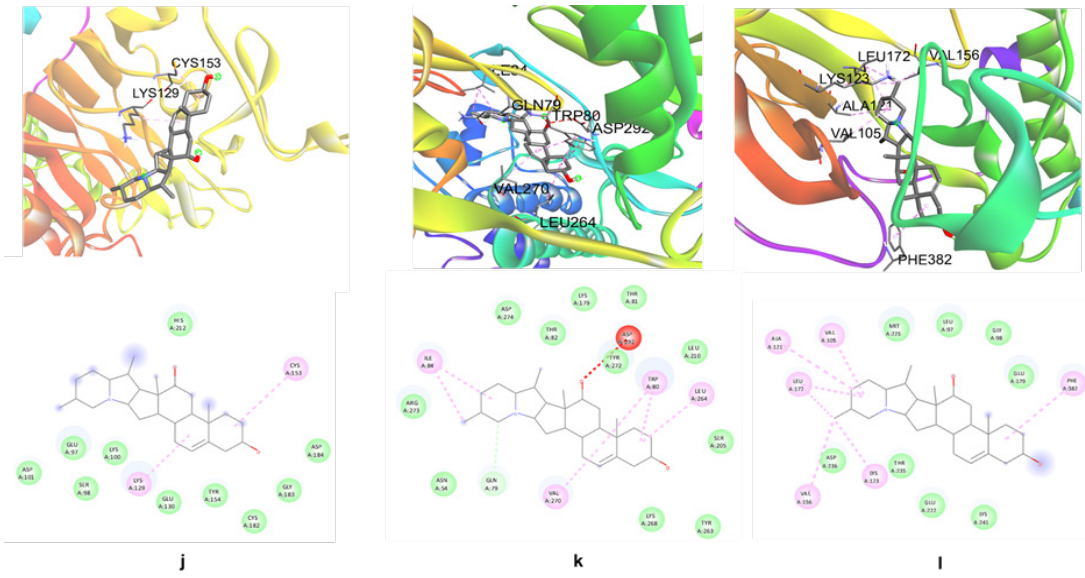


Figure 7. Binding Association of Jervine, Veratramine and Rubijervine with BC Key Targets by Molecular Docking. Analysis by PyRx software illustrates the binding complexes formed between jervine and the following proteins including MTOR, INSR, AKT-1 and RPS6KB1 (a-d), (e-h) represents the binding interactive complexes formed between veratramine and MTOR, INSR, AKT1 and RPS6KB1 proteins (i-l) depicts the interactive complexes formed between rubijervine and the proteins MTOR, INSR, AKT1 and RPS6KB1 targets. The 2D and 3D structural illustrations presented here were visualized using the Biovia Discovery Studio tool.

is depicted in (Figure 7 (a-l), Supplementary Figure 1 and Table 5). Analysis results exhibited that the compounds demonstrate binding affinity towards each target protein, ranging from −6.0 to −13 kcal/mol which ensures reasonably strong interaction with the targets. Specifically noteworthy were that jervine has exhibited the elevated strong binding affinity towards all four target proteins when compared to other two compounds Veratramine and Rubi-jervine. For MTOR, binding affinities ranged from −8.6 to −10 kcal/mol, with jervine showing the strongest (−10 kcal/mol). For INSR, affinities ranged from −8.6 to −8.9 kcal/mol, with jervine again highest (−8.9 kcal/mol). For AKT1, values ranged from −11.8 to −12.9 kcal/mol, with rubijervine strongest (−12.9 kcal/mol). For RPS6KB1, affinities ranged from −9 to −10.6 kcal/mol, with jervine strongest (−10.6 kcal/mol), (Figure 7 (a-l)) explains a visual illustrations presenting both 2D and 3D images of the docking analysis. Moreover, the 2D and 3D pictures that displaying the docking interaction between tamoxifen and the common target proteins, specifically

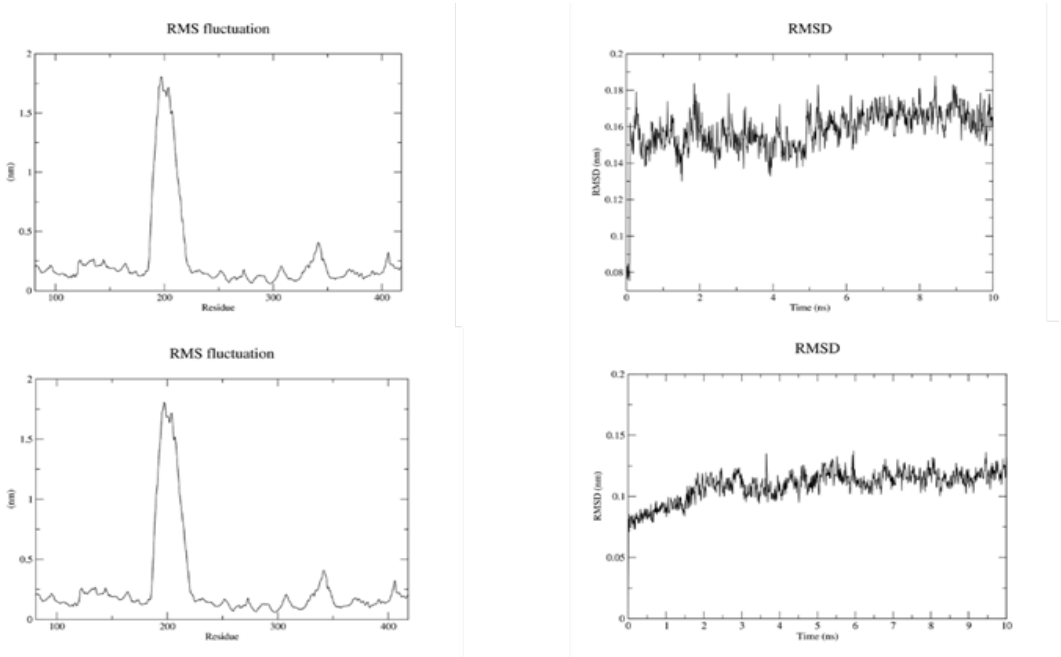


Figure 8. Molecular Dynamic Simulations between AKT-1 and RPS6KB1. Root Mean Square Deviation (RMSD -5 complexes) and Root Mean Square Fluctuation (RMSF) graphs for AKT-1 and RPS6KB1 were displayed here (the molecular dynamics were simulated for 10ns using GROMACS software).

Table. 5. Molecular Docking Results

Compound	Protein	Binding	Conventional Hydrogen Bond	Vanderwaal's force	Carbon Hydrogen/Pi-Alkyl/Pi-Pi Stacked/Pi-Pi T shaped/
Jervine (CID 10098)	MTOR (PDB ID: 7PED)	-10	SER A:2221	GlnA:2223, ARGA:2224, SERA:913, ASNA:2206,THRA:2207,ASPA:907,ASNA:2319,SERA:2350,META:905,ILEA:906,ASPA:907	LysA:2352,ALAA:2210,ARGA:910
	INSR (PDB ID: 5E1S)	-8.9	-	GLYA:1371,ARGA:1372,LEUA:1374,ASPA:169,GLUA:168,ASNA:170,GLUA:725,GLUA:724,SERA:727	PHEA:728,TYRA:171
	AKT-1 (PDB ID: 6HHI)	-12.4	TYR:A 272	LYSA:179,ASPA:292,LEUA:264,GLNA:203,THRA:82,VALA:271,VALA:270,GLNA:79,ASNA:53,ARGA:273	TRPA:80,LYSA:268
	RPS6KB1 (PDB ID: 4L43)	-10.6	-	GLUA:222,LYSA:241,ASPA:236,LYSA:123,VALA:156,LEUA:172,GLYA:98,THRA:235,LYSA:99,LEUA:97,META:225,LYSA:99,PHEA:382	GLUA:173,VALA:105,ALAA:121, LEUA:175
Veratramine (CID 6070)	MTOR (PDB ID: 7PED)	-8.9	-	GLNA:222,LEUA:219,PROA:180,THRA:355,ALAA:211,ARGA:173	ILEA:218,ILEA:177,ALAA:215, ARGA:214
	INSR (PDB ID: 5E1S)	-8.7	-	TYRA:171,GLUA:725,GLUA:724,SERA:727,THRA:731,ASPA:169,GLUA:168	PHEA:728
	AKT-1 (PDB ID: 6HHI)	-11.8	GLNA:203,LYSA:268	LEUA:264,TYR:A272,ASPA:292,ASPA:274,GLYA:294,LYSA:179,THRA:82,VALA:271,GLNA:79,ASNA:53	VALA:270,TRPA:80
	RPS6KB1 (PDB ID: 4L43)	-9	-	GLUA:242, LYSA:99,GLYA:98,LEUA:97,GLUA:173,META:225,TYRA:174,LEUA:175,ALAA:121,THRA:235,LEUA:172,LYSA:123,ASPA:236,GLUA:222	VALA:105,LYSA:241
Rubijervine (CID 253295)	MTOR (PDB ID: 7PED)	-8.6	GLNA:222	PROA:351,SERA:354,ALAA:352,ILEA:177,THRA:355,LEUA:219,ARGA:214,ALAA:215,LEUA:219	ILEA:218,ALAA:211
	INSR (PDB ID: 5E1S)	-8.6	-	HISA:212,GLUA:97,LYSA:100,ASPA:101,SERA:98,GLUA:130,TYRA:154, CYSA:182,GLYA:183,ASPA:184	CYSA:153,LYSA:129
	AKT-1 (PDB ID: 6HHI)	-12.9	-	ASPA:274,LYSA:179,THRA:81,THRA:82,TYRA:272,LEUA:210,SERA:205,ARGA:273,ASNA:54,GLNA:79,LYSA:268,TYRA:263	ASPA:292,ILEA:84,TRPA:80,LEUA:264,VALA:270
	RPS6KB1 (PDB ID: 4L43)	-9.6	-	META:225,LEUA:97,GLYA:98,GLUA:179,ASPA:236,THRA:235,GLUA:222,LYSA:241	-
Tamoxifen (CID 2733526)	MTOR (PDB ID: 7PED)	-7.7	-	GLNA: 2200, GLNA:1937, META: 2199, TYRA:2225, ALA:2226, SERA: 913, ASPA:911, PROA:1940, LEUA:1936, ILEA:1939, PROA:1975, ALA:1971, TYRA: 1974, ILEA: 2228, VALA: 2227, TYRA: 2144, GLYA:2142, ALA:914	ALA:2226, SERA: 913, ASPA:911' PROA-1940, ILEA:1939, PROA: 1975, GLYA:2142
	INSR (PDB ID: 5E1S)	-5.9	-	ARGA:1372,GLUA: 25, LEUA:1374, GLUA:724, ILEA- 1373, PHEA:728, LEUA:1376,LYSA:721, PHEA: 116, ASAN:117	LEUA:1374, PHEA:728, LEUA:1376,
	AKT-1 (PDB ID: 6HHI)	-9.7	-	THR A: 211, ASPA: 292, LEUA: 210, ASPA: 274, TYRA:272, GLNA: 79,LEUA:264,SERA:205,TYRA:263,TRPA:80, VALA:270,LYSA:268,THRA:82, ASNA:54,ILEA:84, VALA: 271, ASNA:53	GLNA:79,LEUA:210, LEUA:264, TRPA:80,VALA:270, ,LYSA:268,
	RPS6KB1 (PDB ID: 4L43)	-7.9	-	LYSA: 241, LEUA: 175, VALA: 105, ASPA: 236, ALAA: 121, VALA: 156, GLYA: 98, LYSA: 123, LEUA: 172, THRA:235,META:225, LEUA: 97, GLYA:178,GLUA:179, SERA: 380, PHEA: 382	LYSA:241, VALA:105, META:225, LEUA:97, PHEA: 382

MTOR, INSR, AKT1, and RPS6KB1, with binding affinities ranging from −5.9 to −9.7 kcal/mol were depicted in (Supplementary figure.1 and Table 5). The binding affinities of jervine seem to be substantially enriched, showing better interactions with the target proteins, even when compared to the binding scores of the standard

medication, tamoxifen. Hence, Jervine can be used as a therapeutic candidate against BC with excellent binding potential.Together, these visual representations support the strength and specificity of the interactions, clearly demonstrating how jervine forms hydrogen bonds with specific active-site residues of the target proteins, showing considerable drug reliability comparable to tamoxifen. Therefore, according to results of binding affinity and its interaction results, jervine shows the highest capacity to connect with four BC targets with affinities ranged from -8.9 to -12.4 kcal/mol. The amino acid interactions and binding capacity between four target proteins and tamoxifen and jervine are shown in Table 5.Moreover, molecular dynamics result suggested that AKT-1/MTOR and RPS6KB1 shows strong stability with jervine for 10ns of simulations as shown in (Figure 8). Hence among the three compounds, jervine can be considered as an effective compound with potential binding affinity towards BC targets.

Discussion

This pioneering study examines the efficacy of bioactive compounds from *Veratrum viride* as therapeutic agents acting against BC. Using BC-related targets from the GeneCards database combined with network pharmacology, molecular docking, and simulation, this study systematically detected the molecular targets and signaling pathways linked to *Veratrum viride*'s anti-breast cancer activity. The analysis underscores the role of key protein targets and their association with multiple BC pathways.

Veratrum viride has been traditionally employed to treat diseases including diabetes, rheumatism, and respiratory disorders, and is also recognized for its notable anticancer and antioxidant activities [7, 8]. Breast cancer (BC) endangers women's health and represents a leading cause of mortality globally [2]. Despite considerable progress in breast cancer (BC) treatment over the past several centuries, most medications still fall short of delivering satisfactory outcomes for patients [3]. Standard breast cancer (BC) chemotherapy faces major challenges, including multidrug resistance (MDR), rapid drug clearance, severe side effects, poor targeting, and low drug concentrations at tumor sites. [30-32]. Thus, the therapies for this dreadful disease seems to be limited Several compounds isolated from many of the Veratrum species including V. Californicum, V. nigrum ,V.parviflorum are reported to have anti-cancer, anti-oxidant and other medicinal activities [8, 33, 34]. Although some research has highlighted the pharmacological properties of *Veratrum viride*, evidence regarding its anticancer potential particularly against BC is currently lacking, with no data or detailed investigations. Furthermore, the multiple targets and signaling pathways through which it exerts its anti-breast cancer effects remain unknown. Thus, this study is the primary one to thoroughly analyse the active ingredients of *Veratrum viride*, predict active ingredients targets and the BC related genes and analyze the potential mechanism of *Veratrum viride* against BC by using a network pharmacology approach, molecular docking and simulation analyses [35, 36].

Network pharmacology (NP) is a systems biology-based approach that verifies drug actions through multi-target interactions rather than a single-target mechanism. This integrative approach involves a combination of molecular biology, pharmacology and science to facilitate drug discovery. By constructing 'disease target protein drug' networks employing existing components such as genes, proteins, and medications, NP provides a comprehensive understanding of drug mechanisms. Once the component-target network is established, further in-depth analysis can be conducted. It also offers a more affordable and quick method of developing new drugs [37]. Many research on BC have applied a network pharmacology-based methodology [30, 35, 38]. Moreover, the several pharmacological actions of bioactive substances show great possibility BC treatment. Network pharmacology, integrated with molecular docking and simulation, enables a systems-level understanding of drug–target interactions and mechanistic pathways for identifying multi-target therapeutics [28]. Further, so far many research studies have been carried out by integrating network pharmacology methodology, molecular docking and simulation to investigate the effects and mechanism of various other plant species against various diseases

For instance, Hu et al performed a study explaining about the therapeutic mechanisms of curcuma in osteosarcoma (OS) which was investigated through network pharmacology and molecular docking analysis. Through public database mining including GeneCards, SwissADME, and PubChem, they found 11 active anticancer compounds connected to curcuma and OS as well as roughly 141 possible therapeutic targets and finally identified 14 hub genes. Employing databases and tools like STRING, Cytoscape (with the MCODE plugin), and DAVID, their findings revealed key targets including AKT1, TNF(Tumor Necrosis Factor), STAT3(Signal Transducer and Activator of Transcription -3), EGFR (Epidermal Growth Factor Receptor), and HSP90AA1 (Heat Shock Protein 90 alpha family class A member 1) which are notably linked with important pathways including PI3K(Phosphoinositide 3 Kinase)/Akt, HIF-1(Hypoxia Inducible Factor-1), ErbB(Erythroblastic leukemia viral oncogene homologue B), and FOXO, all of which play essential roles in OS progression, metastases, and chemoresistance. Further, their findings highlighted important interactions between the designated targets and the central compounds which showcased various affinities towards the targets. Their results taken together implies that curcuma modulates processes including angiogenesis, proliferation, invasion, and treatment resistance in OS by means of a multi-compound, multi-target, multi-pathway strategy, so exerting anti-osteosarcoma effects [36]. There consistency explains the depth of our approach and strengthens the area of our research. Considering their approach similarly, In the current study, after screening of *Veratrum viride* for bioactive compounds, a total of 11 compounds (Table 1) were retrieved by searching the IMPAAT database. The compounds were primarily screened through the evaluation of their pharmacokinetic properties and ADMET analyses using SWISS ADME

analysis. The results revealed that out of 11 compounds, 3 compounds namely Jervine, Veratramine and Rubijervine demonstrated ideal drug characteristics including drug likeness and pharmacokinetic effectiveness (Table 2, 3). Then, to examine the specific target genes of each compound SWISS target prediction tool was utilised and as from the results, overall collectively, 300 potential genes related to jervine, veratramine and rubijervine were identified (Supplementary Table 1, Table 2). Later after removal of duplicates, 106 unique genes were identified as finalised target genes (Supplementary Table 3) and also using Genecard database about 2657 genes related to BC was selected (Supplementary Table 4).Afterwards, the compound disease target network for the compounds jervine, veratramine and rubijervine in BC treatment was constructed based upon the intersection of compounds and BC target networks with 8 overlapping genes *AKT2, AKT1, AKT3, JAK1, PRKG1,ROCK2, RPS6KB1, INSR* (Figure 2(a&b)). Further,the interaction between these 8 target genes and its relation with each of the three compounds (jervine, veratramine and rubijervine) DPI network was established using STITCH and visualised using Cytoscape and the results revealed a network of 21 nodes and 106 edges (Figure 2c). Further,the PPI network was conducted based upon four main ranking criteria namely Radiality, Closeness, Degree and MCC. The results revealed a total of six hub gene targets *MTOR,INSR,FOXO3,FOXO1, AKT-1* and *RPS6KB-1* (Figure 3(a-d)) among them common four target genes *MTOR,INSR, AKT-1* and *RPS6KB-1* (Figure 3e) for those three anti-BC compounds jervine, veratramine and rubijervine were identified. It was evident that all these targets including MTOR,INSR, AKT-1 and RPS6KB-1 performs an significant role in BC pathogenesis and these proteins are largely interconnected through the PI3K/AKT/mTOR signaling pathway, which plays a ideal role in managing breast cancer (BC) cell metabolism, proliferation, and survival. Dysregulation of this pathway is frequently associated with tumor progression, therapy resistance, and poor clinical outcomes [39].

The mTOR (Mammalian Target of Rapamycin) protein is a crucial downstream effector in the PI3K/AKT pathway that promotes the growth, survival, and treatment resistance of BC cells. Its abnormal activation, frequently brought on by PTEN loss or PIK3CA mutations, which lowers the prognosis for patients with breast cancer (BC) [40]. AKT, or protein kinase B (PKB), is an another key regulator in the PI3K pathway which also aids in controlling cell growth, survival, and metabolism.Its hyperactivation drives oncogenesis and therapy resistance in HR+ breast cancer (BC) and moreover reports also highlighted that in patients with HR+ cases AKT gets altered and also currently AKT inhibitors like capivasertib and fulvestrant were also in use as promising target for combined endocrine or chemotherapy [41]. RPS6KB1, a downstream target of mTOR, facilitates protein synthesis, cell growth, and proliferation [42]. Its amplification in estrogen receptor (ER)-positive breast tumors has been identified as a prognostic marker associated with increased metastatic potential [43]. INSR (The insulin receptor) an important upstream mediator of PI3K/AKT pathway which regulates cell growth, survival and metabolism. It initiates a series of events in response to insulin or insulin-like growth factors that activates PI3K which phosphorylates AKT. Majorly, in estrogen receptor-positive (ER+) subtypes of BC, deregulation of this PI3K/AKT related pathway leads to tumor growth and resistance to endocrine therapies [44].

Followed by the topological degree analysis, to clarify and brief the potential importance of the common targets connected with jervine, veratramine, and rubijervine in breast cancer (BC) (Table 4), comprehensive mRNA expression and survival analyses were performed using the GEPIA database. For instance, in a study carried out by Fariha et al, gene expression and survival analyses of the WNT gene family in BC were carried out. In which WNT2 and WNT11 expression levels were elevated representing better overall survival in patients with BC, while WNT7B expression was reduced showing worse prognosis. Moreover, The UALCAN study consistently showed elevated levels of WNT2 and WNT7B and reduced levels of WNT11 across cancer stages and subtypes. Together, these results imply that WNT2 and WNT11 might be good prognostic markers while WNT7B might be an oncogenic factor in the development of BRCA [45]. In accordance with these findings, the present study demonstrated that comparative mRNA expression analysis between BC tissues and normal samples revealed significant up regulation of MTOR, AKT1, INSR and RPS6KB1 gene levels (Figure 4a). Furthermore, results from the survival analysis revealed a strong correlation between the elevated expression of *MTOR, AKT1, INSR,* and *RPS6KB1* genes and poor patient survival outcomes (Figure 4b). The stage expression levels were analysed and the results showed higher levels at advanced stages suggesting its role as potential diagnostic marker in BC tumorogenesis (Figure 5).

Further, Important target-related enrichment analyses of the KEGG and GO pathways were also carried out. Integrating pathway enrichment analyses and gene ontology (GO) is essential for elucidating the impactful functional importance of the genes associated with breast cancer (BC), especially those related to Jervine, Veratramine and Rubijervine. Using datasets from g: Profiler, the analysis identified the top five significantly enriched GO terms across biological processes, cellular components, molecular functions, as well as KEGG pathways. Based on the results from GO terms, it was evident that the pharmacological effects of Jervine, Veratramine and Rubijervine against BC happened by simultaneously activating BP, CC and MF (Figure 6a) and from the KEGG enrichment analysis, it was found that the three alkaloid compounds including jervine, veratramine and rubijervine could potentially suppress BC through multiple pathways, including pathways in breast cancer (BC) etc which are depicted in (Figure 6b). Moreover, in accordance with the results of mRNA expression and survival analysis depicted in Figure 4b, the KEGG pathway analysis revealed that the AMPK, HIF-1, and FOXO signaling pathways were highly enriched, indicating elevated expression levels of the AKT, MTOR, INSR, and RPS6KB1 genes involved in these

pathways, Notably, as key regulatory genes involving in AMPK -mTOR signaling PI3K-AKT signaling pathways, their dysregulation may contribute to impaired feedback inhibition and enhanced oncogenic signaling [42]. Moreover, AMPK (AMP activated Protein Kinase), a key metabolic sensor which links energy homeostasis to cellular signaling and has been implicated in cancer regulation. Its expression is higher in triple-negative breast cancer (TNBC) than in non-TNBC, correlating with advanced TNM stage, metastasis, and poor survival outcomes [46] and among the many important facets of BC biology, the HIF-1 pathway also essential for epithelial–mesenchymal transition (EMT), invasion and metastasis, and resistance to chemotherapy radiation [47]. .FOXOs are a key resultant component of the PI3K/Akt pathway and are subject to its negative regulation [48]. They are the transcription factors which control important biological functions like growth, metabolism, stem cell maintenance, and longevity. They acts as tumor suppressors by regulating genes involved in angiogenesis, apoptosis, senescence, proliferation, and metastasis, Particularly, it has been reported that FOXO1 and FOXO3 have a strong correlation with the development of metastases in cancers like kidney, pancreatic, and BC [49]. Later, molecular docking analysis was performed to validate the potential mechanism of Jervine, Veratramine,Rubijervine and Tamoxifen (for comparison) against 4 major targets named (MTOR, INSR, RPS6KB1 and AKT-1) depending on the variables of compound genes pathway network. Results revealed that compounds of *Veratrum viride* can stably bind with core targets of BC with jervine displaying the highest affinity across all six highlighting its multitargeting potential in treatment of BC (Figure 7(a-l)) and also jervine displayed high binding affinity scores with the targets when comparing with the other compounds and also in comparison with the tamoxifen drug the binding interaction scores of jervine seems to be relatively higher (Table 5). Moreover, effective hydrogen bonding within the active site enhances the stability, specificity, and overall docking score of the ligand–protein complex [50]. As depicted in Table 5, Jervine, Veratramine, Rubijervine, and Tamoxifen were observed to bind at specific sites corresponding to their target proteins. Notably, Jervine was found to interact with the SERA: 2221 residue of mTOR and the TYRA: 272 residue of AKT1, while Veratramine showed binding at the GLNA:203 and LYSA:268 residues of AKT1. In contrast, Rubijervine exhibited interaction with the GLNA:222 residue of the mTOR protein. Relatively, Molecular dynamics also shows significant stability towards jervine and key targets (Figure 8). Together, in the recent network pharmacology, this study identified that the V.viride based active compounds, its possible targets and relatively related signaling pathways for the therapy of BC, thereby offering a theoretical basis for further experimental investigations. Data mining from multiple databases is the primary source of information into BC treatment mechanisms due to network pharmacology's limitations. Despite curation, inconsistencies often arise from diverse data sources and experimental variations. As this study is based on computational analysis, experimental validation is lacking, further studies on these compounds, targets, and pathways are needed to confirm the therapeutic potential and mechanisms of *V. viride* against BC.

In conclusion, this study delivers a scientific basis for exploring multicomponent, multi-target therapies and novel anti-breast cancer targets. *Veratrum viride* was investigated through network pharmacology and in silico analysis to assess its phytochemical potential against breast cancer (BC). Network analysis revealed that *V. viride* contains compounds acting on multiple disease-related pathways. Notably, docking and simulation studies identified jervine as a potent compound targeting six key BC-related proteins (MTOR, INSR, FOXO1, FOXO3, RPS6KB1, and AKT1). Jervine also showed favorable drug-likeness, pharmacokinetics, low predicted toxicity, and good oral bioavailability. These findings highlight its promise as a therapeutic agent. However, further In vitro and In vivo studies are needed to validate these results, and more phytochemical and pharmacological research is justified.

Author Contribution Statement

Anupriya Eswaran & Sathanraj Natarajan-Preparation of methodology, experimentation, manuscript writing. Selvaraj Jayaraman-Idea sharing, critical thinking, correction. Vishnupriya Veeraraghavan-Conceptualization, Manuscript review and correction.

Acknowledgements

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India Panneerselvam and Sathanraj Natrajan, Research scholars, Saveetha dental college and Hospitals for their help and guidance.

Conflict of interest

None.

References

1. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018;5(2):77-106. https://doi.org/10.1016/j.gendis.2018.05.001.
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(3):209-49. https://doi.org/10.3322/caac.21660.
3. McCarthy AM, Barlow WE, Conant EF, Haas JS, Li CI, Sprague BL, et al. Breast cancer with a poor prognosis diagnosed after screening mammography with negative results. JAMA Oncol. 2018;4(7):998-1001. https://doi.org/10.1001/jamaoncol.2018.0352.
4. Trayes KP, Cokenakes SEH. Breast cancer treatment. Am Fam Physician. 2021;104(2):171-8.
5. Adeleye OA, Bamiro OA, Bakre LG, Odeleye FO, Adebowale MN, Okunye OL, et al. Medicinal plants with potential inhibitory bioactive compounds against coronaviruses. Adv

Pharm Bull. 2022;12(1):7-16. https://doi.org/10.34172/apb.2022.003.
6. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr. 2023;11(6):2500-29. https://doi.org/10.1002/fsn3.3308.
7. Dirks ML, Seale JT, Collins JM, McDougal OM. Review: Veratrum californicum alkaloids. Molecules. 2021;26(19). https://doi.org/10.3390/molecules26195934.
8. Seale JT, McDougal OM. Veratrum parviflorum: An underexplored source for bioactive steroidal alkaloids. Molecules. 2022;27(16). https://doi.org/10.3390/molecules27165349.
9. Seale JT, Carpenter JE, Eisenstat MD, Kiernan EA, Morgan BW, Nogee DP, et al. Veratrum parviflorum poisoning: Identification of steroidal alkaloids in patient blood and breast milk. Clin Toxicol (Phila). 2022;60(12):1309-17. https://doi.org/10.1080/15563650.2022.2132166.
10. Jaffe AM, Gephardt D, Courtemanche L. Poisoning due to ingestion of veratrum viride (false hellebore). J Emerg Med. 1990;8(2):161-7. https://doi.org/10.1016/0736-4679(90)90226-l.
11. El sayed ka, mcchesney jd, halim af, zaghloul am, lee is. A study of alkaloids in veratrum viride aiton. Int j pharmacogn.1996;34:161–73.
12. Guo Y, Fang R, Jiao Y, Liu J, Lu JT, Luo T. Divergent syntheses of complex veratrum alkaloids. Nat Commun. 2024;15(1):7639. https://doi.org/10.1038/s41467-024-52134-7.
13. Shang Y, Du Q, Liu S, Staadler M, Wang S, Wang D. Antitumor activity of isosteroidal alkaloids from the plants in the genus veratrum and fritillaria. Curr Protein Pept Sci. 2018;19(3):302-10. https://doi.org/10.2174/1389203718666170106103747.
14. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, et al. Imppat: A curated database of indian medicinal plants, phytochemistry and therapeutics. Sci Rep. 2018;8(1):4329. https://doi.org/10.1038/s41598-018-22631-z.
15. Daina A, Michielin O, Zoete V. Swissadme: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717. https://doi.org/10.1038/srep42717.
16. Daina A, Michielin O, Zoete V. Swisstargetprediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357-w64. https://doi.org/10.1093/nar/gkz382.
17. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The genecards suite: From gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016;54:1.30.1-1..3. https://doi.org/10.1002/cpbi.5.
18. Fonseka P, Pathan M, Chitti SV, Kang T, Mathivanan S. Funrich enables enrichment analysis of omics datasets. J Mol Biol. 2021;433(11):166747. https://doi.org/10.1016/j.jmb.2020.166747.
19. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. Stitch 5: Augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 2016;44(D1):D380-4. https://doi.org/10.1093/nar/gkv1277.
20. Jayaraman S, Veeraraghavan VP, Natarajan SR, Jasmine S. Exploring the therapeutic potential of curcumin in oral squamous cell carcinoma (hsc-3 cells): Molecular insights into hypoxia-mediated angiogenesis. Pathol Res Pract. 2024;254:155130. https://doi.org/10.1016/j.prp.2024.155130.
21. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. Gepia: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-w102. https://doi.org/10.1093/nar/gkx247.
22. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. David: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 2022;50(W1):W216-w21. https://doi.org/10.1093/nar/gkac194.
23. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023;13(1):13398. https://doi.org/10.1038/s41598-023-40160-2.
24. Dallakyan S, Olson AJ. Small-molecule library screening by docking with pyrx. Methods Mol Biol. 2015;1263:243-50. https://doi.org/10.1007/978-1-4939-2269-7_19.
25. Rakhshani H, Dehghanian E, Rahati A. Enhanced gromacs: Toward a better numerical simulation framework. J Mol Model. 2019;25(12):355. https://doi.org/10.1007/s00894-019-4232-z.
26. Rosignoli S, Paiardini A. Boosting the full potential of pymol with structural biology plugins. Biomolecules. 2022;12(12). https://doi.org/10.3390/biom12121764.
27. Kagami L, Wilter A, Diaz A, Vranken W. The acpype web server for small-molecule md topology generation. Bioinformatics. 2023;39(6). https://doi.org/10.1093/bioinformatics/btad350.
28. Rizzuti B. Molecular simulations of proteins: From simplified physical interactions to complex biological phenomena. Biochim Biophys Acta Proteins Proteom. 2022;1870(3):140757. https://doi.org/10.1016/j.bbapap.2022.140757.
29. Wali AF, Talath S, El Tanani M, Rashid Rangraze I, Babiker R, Shafi S, et al. Pi3k/akt/mtor pathway in breast cancer pathogenesis and therapy: Insights into phytochemical-based therapeutics. Nutr Cancer. 2025;77(9):938-58. https://doi.org/10.1080/01635581.2025.2521884.
30. Zhang X, Shen T, Zhou X, Tang X, Gao R, Xu L, et al. Network pharmacology based virtual screening of active constituents of prunella vulgaris l. And the molecular mechanism against breast cancer. Sci Rep. 2020;10(1):15730. https://doi.org/10.1038/s41598-020-72797-8.
31. Oh KK, Adnan M, Cho DH. Network pharmacology-based study to uncover potential pharmacological mechanisms of korean thistle (cirsium japonicum var. Maackii (maxim.) matsum.) flower against cancer. Molecules. 2021;26(19). https://doi.org/10.3390/molecules26195904.
32. Adnan M, Jeon BB, Chowdhury MHU, Oh KK, Das T, Chy MNU, et al. Network pharmacology study to reveal the potentiality of a methanol extract of caesalpinia sappan l. Wood against type-2 diabetes mellitus. Life (Basel). 2022;12(2). https://doi.org/10.3390/life12020277.
33. Kee JY, Han YH, Mun JG, Um JY, Hong SH. Pharmacological effect of prohibited combination pair panax ginseng and veratrum nigrum on colorectal metastasis in vitro and in vivo. J Ethnopharmacol. 2018;220:177-87. https://doi.org/10.1016/j.jep.2018.03.036.
34. Turner MW, Rossi M, Campfield V, French J, Hunt E, Wade E, et al. Steroidal alkaloid variation in veratrum californicum as determined by modern methods of analytical analysis. Fitoterapia. 2019;137:104281. https://doi.org/10.1016/j.fitote.2019.104281.
35. Mir WR, Bhat BA, Kumar A, Dhiman R, Alkhanani M, Almilaibary A, et al. Network pharmacology combined with molecular docking and in vitro verification reveals the

therapeutic potential of delphinium roylei munz constituents on breast carcinoma. Front Pharmacol. 2023;14:1135898. https://doi.org/10.3389/fphar.2023.1135898.
36. Hu M, Yan H, Li H, Feng Y, Sun W, Ren Y, et al. Use of network pharmacology and molecular docking to explore the mechanism of action of curcuma in the treatment of osteosarcoma. Sci Rep. 2023;13(1):9569. https://doi.org/10.1038/s41598-023-36687-z.
37. Zhai Z, Tao X, Alami MM, Shu S, Wang X. Network pharmacology and molecular docking combined to analyze the molecular and pharmacological mechanism of pinellia ternata in the treatment of hypertension. Curr Issues Mol Biol. 2021;43(1):65-78. https://doi.org/10.3390/cimb43010006.
38. Qayoom H, Alkhanani M, Almilaibary A, Alsagaby SA, Mir MA. A network pharmacology-based investigation of brugine reveals its multi-target molecular mechanism against breast cancer. Med Oncol. 2023;40(7):202. https://doi.org/10.1007/s12032-023-02067-w.
39. Costa RLB, Han HS, Gradishar WJ. Targeting the pi3k/akt/mtor pathway in triple-negative breast cancer: A review. Breast Cancer Res Treat. 2018;169(3):397-406. https://doi.org/10.1007/s10549-018-4697-y.
40. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The pi3k pathway in human disease. Cell. 2017;170(4):605-35. https://doi.org/10.1016/j.cell.2017.07.029.
41. Martorana F, Motta G, Pavone G, Motta L, Stella S, Vitale SR, et al. Akt inhibitors: New weapons in the fight against breast cancer? Front Pharmacol. 2021;12:662232. https://doi.org/10.3389/fphar.2021.662232.
42. Daragmeh J, Barriah W, Saad B, Zaid H. Analysis of pi3k pathway components in human cancers. Oncol Lett. 2016;11(4):2913-8. https://doi.org/10.3892/ol.2016.4309.
43. Holz MK. The role of s6k1 in er-positive breast cancer. Cell Cycle. 2012;11(17):3159-65. https://doi.org/10.4161/cc.21194.
44. Fox EM, Kuba MG, Miller TW, Davies BR, Arteaga CL. Autocrine igf-i/insulin receptor axis compensates for inhibition of akt in er-positive breast cancer cells with resistance to estrogen deprivation. Breast Cancer Res. 2013;15(4):R55. https://doi.org/10.1186/bcr3449.
45. Fariha FTJ, Fuad M, Saha CS, Hossen S, Hossain MJ. Comprehensive bioinformatics analysis reveals prognostic significance and immunological roles of wnt gene family in breast cancer. Sci Rep. 2025;15(1):34490. https://doi.org/10.1038/s41598-025-13315-6.
46. Cao W, Li J, Hao Q, Vadgama JV, Wu Y. Amp-activated protein kinase: A potential therapeutic target for triple-negative breast cancer. Breast Cancer Res. 2019;21(1):29. https://doi.org/10.1186/s13058-019-1107-2.
47. Liu ZJ, Semenza GL, Zhang HF. Hypoxia-inducible factor 1 and breast cancer metastasis. J Zhejiang Univ Sci B. 2015;16(1):32-43. https://doi.org/10.1631/jzus.B1400221.
48. Shi YY, Meng XT, Xu YN, Tian XJ. Role of foxo protein's abnormal activation through pi3k/akt pathway in platinum resistance of ovarian cancer. J Obstet Gynaecol Res. 2021;47(6):1946-57. https://doi.org/10.1111/jog.14753.
49. Jiramongkol Y, Lam EW. Foxo transcription factor family in cancer and metastasis. Cancer Metastasis Rev. 2020;39(3):681-709. https://doi.org/10.1007/s10555-020-09883-w.
50. Vaidyanathan R, Sreedevi SM, Ravichandran K, Vinod SM, Krishnan YH, Babu LK, et al. Molecular docking approach on the binding stability of derivatives of phenolic acids (dpas) with human serum albumin (hsa): Hydrogen-bonding versus hydrophobic interactions or combined influences? Jcis Open. 2023;12:100096.