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Network pharmacology and computational dissection of *Solanum trilobatum* bioactives reveals modulation of steroid receptor associated signalling in breast cancer

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Abstract

Background Breast cancer is the most common form of malignancy in women worldwide. Although chemotherapy has been improved, the major drawbacks demand alternative treatment plans. Bioactives based on plants have become a key focus of interest as complementary agents and *Solanum trilobatum* has been known to possess multiple pharmacological activities that include, antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects. The molecular paths of action of its anticancer effect, however, is mostly unexplored.

Methods An integrative in silico strategy combining network pharmacology, molecular docking, molecular dynamics (MD) simulations, and binding free energy calculations was employed to elucidate the anticancer mechanisms of *S. trilobatum* bioactives. Phytoconstituents were mined from curated databases and screened based on drug-likeness, ADMET properties, and predicted anti-neoplastic activity. Protein protein interaction networks, KEGG pathway enrichment, and Gene Ontology analyses were performed to identify key pathways and hub genes. Selected bioactives were docked against oncogenic targets using AutoDock Vina and Schrödinger Glide. The most promising protein ligand complexes were further validated through long-timescale MD simulations and MMPBSA binding free energy analysis.

Results The enrichment of the analysis by KEGG pathways demonstrated the PI3K-Akt signalling pathway as the pathway that was massively altered. Network analysis identified AKT1, TP53, TNF, STAT3, MYC, IL6, HIF1A, EGFR, CTNNB1, and CASP3 as hub genes. Molecular docking of rheochrysin, hypoxiside, cytochalasin and 4- p-coumatoylquinic acid to oncogenic proteins, HRAS, KRAS, NCOA3 (a nuclear coactivator of steroid receptors), PGR, and PIK3CB oncogenes was observed using AutoDock 4.2 and Schrodinger Glide with binding affinities displaying strong binding. Molecular dynamics simulations further confirmed stable and sustained interactions, particularly between KRAS–4-p-coumaroylquinic acid and TP53–Hypoxoside A complexes.

Conclusion These findings provide mechanistic insights into the anticancer potential of *S. trilobatum*, supporting its role as a promising source of bioactive compounds capable of modulating steroid receptor–associated signalling networks in breast cancer therapy.

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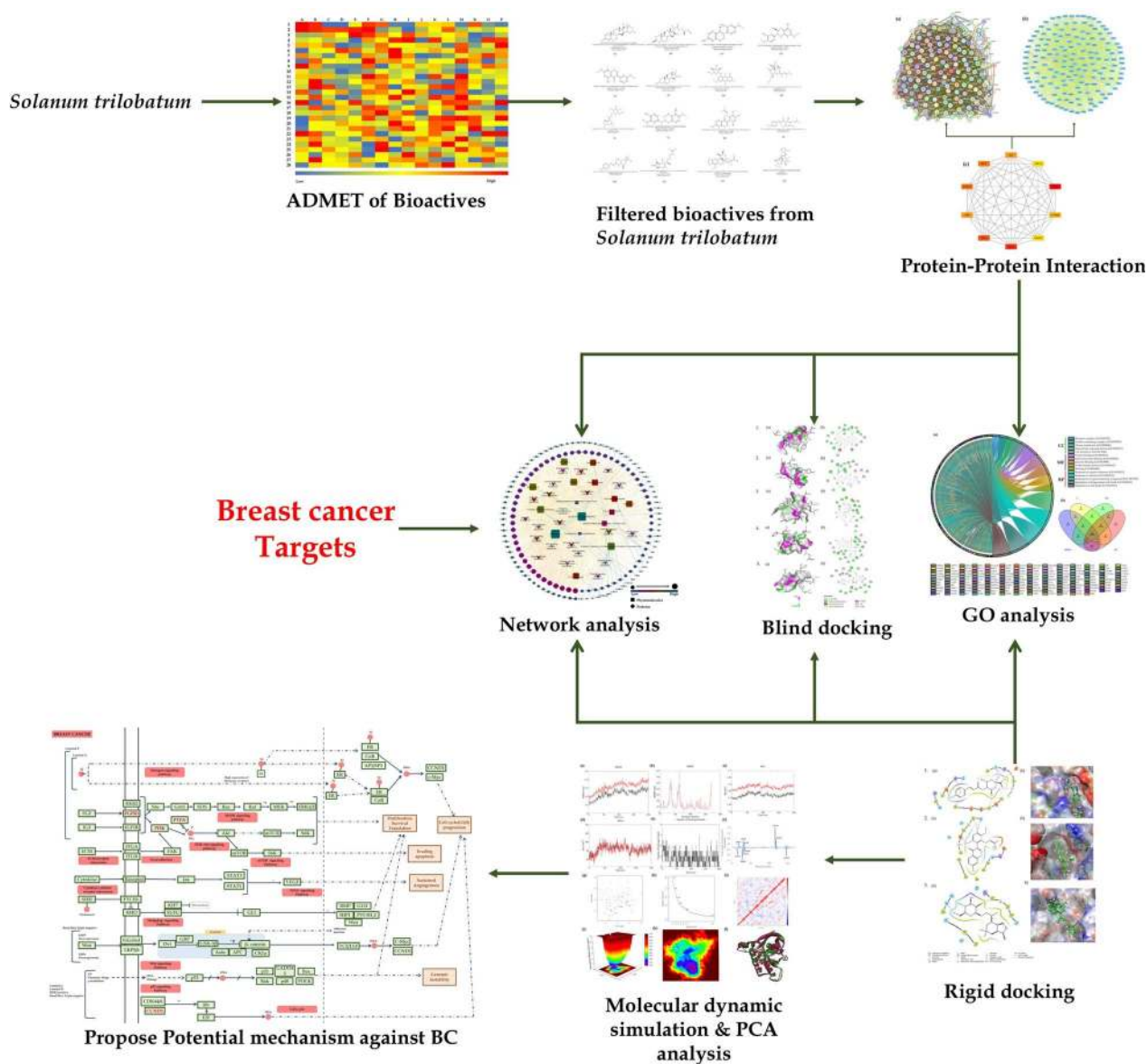
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Keywords Breast cancer, Molecular docking, Molecular simulation, Network pharmacology, Steroid receptors, *Solanum trilobatum*

Graphic abstract



1 Introduction

Breast cancer is the most prevalent malignancy among women worldwide and remains the leading cause of cancer-related mortality in women across 140 out of 184 countries [1]. Globally, approximately 1.5 million women are diagnosed with breast cancer annually [2]. It is a heterogeneous disease characterized by diverse

morphological features, biological behaviours, clinical courses, and prognoses [3]. Based on the tissue of origin, breast cancers are broadly classified into carcinomas and sarcomas [4]. Furthermore, based on pathological features and invasiveness, breast cancer is categorized into three major groups *i.e.*, non-invasive (or in situ), invasive, and metastatic breast cancer [5]. The classification of breast

cancer into specific molecular subtypes, as a result of gene expression profiling, has shown a vast difference in prognosis and treatment targets. Some of these subtypes are luminal A, luminal B, HER2-positive and triple-negative breast cancer (TNBC) [6]. There are numerous risk factors that lead to the development of breast cancer, which are either modifiable (obesity, sedentary lifestyle, exposure to exogenous hormones) or non-modifiable (genetic inclination and aging) [4].

The treatment options used at the present date to treat breast cancer are surgery, radiology, immunotherapy, hormone, and chemotherapy [7, 8]. The first treatment modality continues to be chemotherapy which is informed by genetic expression profiling. It does however also pose much challenge, which in its turn can be called a two-sided sword, because it has on the other side effects that have been reported to be adverse [9]. As an example, aromatase inhibitors like letrozole, anastrozole, and exemestane are associated with such side effects as hot flushes, depression, anxiety, and memory impairment. Selective estrogen receptor modulators (SERMs) like tamoxifen and raloxifene [10] pose risks of deep vein thrombosis (DVT), pulmonary embolism (PE), and retinal vein thrombosis [11]. Moreover, doxorubicin and daunorubicin are examples of anthracycline linked with cardiotoxicity [12], and paclitaxel and docetaxel are related to chemotherapy induced peripheral neuropathy (CIPN) [13].

Given the limitations of conventional treatment, the interest to plant-derived compounds in breast cancer treatment has increased. It has been historically known that medicinal plants were used as early as more than 5,000 years ago and traditional systems of herbs such as the Chinese herbal medicine and Ayurveda provide a rich variety of botanical formulations. The relationship between herbal preparations and anticancer potential has evoked potential activities including anticancer effects using decoctions, patented formulations, and bioactive extracts [14]. Ayurvedic herbs such as *Gmelina arborea*, *Commiphora mukul*, *Foeniculum vulgare*, *Brassica juncea*, *Vitis vinifera*, and *Withania somnifera* exhibit anticancer properties by suppressing nuclear factor-kappa B (NFkB). Besides, *Curcuma longa* and *Zingiber officinale* were also discovered to suppress Bcl-2, whereas Aloe vera also demonstrated the possibility to suppress HER2 receptors [15]. It is said that these herbal compounds are less toxic, safer, and less resistant than hormonal targeting agents [16].

One example of a Solanaceae family, with alkaloids and terpenoid is *Solanum trilobatum* containing steroid-modulating structurally or functionally related phytochemicals identified in related species. Traditionally known for its medicinal use of different parts such

as leaves, flowers, fruits, and roots it is commonly referred to as climbing brinjal or nightshade [17, 18]. It is scientifically shown that *S. trilobatum* has antioxidant [19, 20], hepatoprotective [21], anticancer [22], anti-inflammatory [23], and antimicrobial properties [24]. Moreover, the silver nanoparticles prepared using *S. trilobatum* fruit extract exhibit cytotoxicity effects on the MCF-7 cells of breast cancer [25]. Moreover, the silver nanoparticles prepared using *S. trilobatum* fruit extract exhibit cytotoxicity effects on the MCF-7 cells of breast cancer [26].

The molecular mechanisms responsible of the anticancer activity of *S. trilobatum* are not well studied despite its promising pharmacological effects. The present study aims to understand the mode of action of *S. trilobatum* bioactives in the treatment of breast cancer in an integrative manner incorporating a network pharmacology, molecular docking and molecular dynamics simulations.

Despite the traditional medicinal relevance of *S. trilobatum*, systematic studies elucidating its molecular mechanisms against breast cancer remain limited. In particular, the specific phytochemicals responsible for its anticancer activity, their molecular targets, and the associated signalling pathways have not been comprehensively characterised. Hence, the present study was designed to identify potential bioactive phytochemicals and key molecular targets of *S. trilobatum* against breast cancer using an integrated in silico framework, encompassing drug-likeness screening (DLS), ADMET profiling, gene ontology analysis, network pharmacology, molecular docking, and molecular dynamics simulations. The present study results have shown that several important targets that have been regulated by *S. trilobatum* bioactives, includes NCOA3, PGR, ESR-linked signalling components, IGF1R, PI3KCB, and TP53, which collectively influence steroid hormone receptor stimulation and resultant transcriptional programs. The network analysis showed enrichment of the PI3K-Akt pathway, which is a key signalling axis that is functionally linked with estrogen and progesterone receptor activity. Moreover, molecular docking and dynamics supported stable interactions of chosen bioactives with KRAS, TP53, NCOA3, PGR, and IGF1R, hence, represent mechanistic support that *S. trilobatum* phytoconstituents have the ability to affect steroid receptor-associated signal transduction. The findings prove that *S. trilobatum* exerts some of its anticancer effects by regulating steroid-regulated signaling pathways.

2 Materials and methods

2.1 Network pharmacology

2.1.1 Mining of phytoconstituents

Synonyms of the plant were obtained from WFO (<https://wfoplantlist.org/>) and Dr. Dukes (<https://phytochem.nal.usda.gov/>) databases. A list of reported phytoconstituents from all the synonyms of the plant were retrieved from the Chemical Entities of Biological Interest (ChEBI; <https://www.ebi.ac.uk/chebi/>) and Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPATT; <https://cb.imsc.res.in/imppat/>) database. The PubChem CID, molecular formula, molecular weight, canonical SMILES and InChI key for the identified bioactives was obtained from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) database.

2.1.2 Drug-likeness, ADMET profile, and biological spectra of bio-actives

Canonical SMILES for all identified phytomolecules were retrieved and used as input for subsequent in silico screening. The drug-likeness score (DLS) of each compound was calculated using MolSoft (<https://molsoft.com/mprop/>). A positive DLS value was considered indicative of a drug-like and potentially developable molecular profile, and only compounds meeting this criterion were retained for further evaluation. Pharmacokinetic and toxicity properties were assessed using admetSAR 2.0 (<https://lmmd.ecust.edu.cn/admetSar1/predict/>). The predicted ADMET parameters included blood–brain barrier permeability, human intestinal absorption, Caco-2 permeability, P-glycoprotein (P-gp) inhibition and substrate potential, renal organic cation transporter interaction, cytochrome P450 enzyme substrate profiles (CYP2C9, CYP2D6, CYP3A4), CYP inhibition (CYP2C9, CYP2D6, CYP2C19, CYP3A4), CYP inhibitor promiscuity, human ether-à-go-go-related gene (hERG) inhibition, AMES toxicity, carcinogenicity, fish toxicity, Tetrahymena pyriformis toxicity, honey bee toxicity, biodegradability, acute oral toxicity, and oral solubility. In addition, Lipinski's Rule of Five was applied, and compounds showing zero Lipinski violations were selected to ensure favourable oral bioavailability characteristics. The biological activity spectra of the screened compounds were predicted using the PASS online server (Way2Drug) (<https://www.way2drug.com/passonline/>). Compounds predicted to exhibit antineoplastic activity, specifically related to breast cancer, were shortlisted based on a probability of activity (Pa) value greater than 0.7, indicating a high likelihood of biological relevance.

2.1.3 Target fishing

The targets regulated by bioactives of *S. trilobatum* were retrieved from DIGEP-Pred (<https://www.way2drug.com/digep-pred/>), SwissTargetPrediction (www.swiss-targetprediction.ch/), and Bindingdb (<https://www.bindingdb.org/>). The targets involved in the pathogenesis of breast cancer were fetched from TTD (Keyword: Breast cancer), CTD (Keyword: Breast Neoplasms), and DisGeNET databases (keywords: *Breast cancer*, C0006142; *Invasive breast cancer*, C0853879; *Triple-Negative Breast Cancers*, C3539878; *Breast cancer stage IV*, C0278488; *Estrogen receptor-positive breast cancer*, C2938924; *ER-negative breast cancer*, C4733092; *Breast cancer (ductal)*, C1527349; *Advanced breast cancer*, C349591; *Carcinoma of breast*, C0678222; *Triple-Negative Breast Carcinoma*, C4722518). The identified targets modulated by bioactives were further matched with the targets of breast cancer to identify potential common targets.

2.1.4 Network construction and Gene Ontology (GO) analysis

A protein–protein interaction (PPI) network was constructed using the STRING [27] ver. 11.5 (<https://string-db.org/>) based on the matched molecular targets. To gain insights into the functional implications of the identified targets, KEGG pathway enrichment analysis was performed. The resulting bioactive-regulated pathways were further integrated with their associated proteins to build a comprehensive pathway-protein interaction network using Cytoscape [28] ver. 3.9.0. The network was examined in the form of a directed graph with node size and color associated with a number of edges. There was an elaborate network topology analysis based on degree distribution of the nodes and betweenness centrality, eccentricity, neighbourhood connectivity, in-degree and out-degree distribution to determine network robustness and relevancy of interest [29]. Also, Gene ontology (GO) annotations such as Cellular Component (CC), Molecular Function (MF) and Biological Process (BP) were obtained at the STRING database to perform the functional categorization of the targets [30]. Also, Gene ontology (GO) annotations such as Cellular Component (CC), Molecular Function (MF) and Biological Process (BP) were obtained at the STRING database to perform the functional categorization of the targets [29], was created to create a visual representation of the top five terms of each GO category and gave an intuitive description of the multifunctional roles of the bioactive-regulated targets.

2.2 In silico molecular docking

A two-step molecular docking strategy was employed to ensure both comprehensive screening and precise binding analysis. Initially, blind docking was performed using AutoDock Vina to explore the entire protein surface without predefined binding constraints, allowing unbiased identification of favourable binding pockets for structurally diverse phytomolecules. Subsequently, site-specific docking was carried out using Schrödinger Glide, wherein active site residues were identified using Discovery Studio and CASTp based on pocket geometry and residue accessibility. The identified key residues were used to define the docking grid, enabling accurate pose prediction and reliable binding affinity estimation. The docking process was initiated by preparation of the ligand, which involved minimisation of energy, after which the target protein was prepared and analysed in a docking manner.

The chemical structures of the ligands, as the 2D structure, were found in the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in the form of the.sdf file. Minimization of these structures was done using the MMFF97 force field and finally changed to.pdbqt format through BOVIA Discovery Studio Visualizer (<https://discover.3ds.com/discovery-studio-visualizer-download>) so that they can be compatible with docking tools. Appropriate protein standards were determined by reference to the GeneCards database (<https://www.genecards.org/>) and prepared in the same way as the bioactive ligands.

The protein structures, as well as their corresponding standards and resulting complexes, were also converted into.pdbqt format using the Discovery Studio Visualizer. To obtain the target protein structures, sequence data were initially queried from the UniProt database (<https://www.uniprot.org/>), and available 3D structures were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>). For targets without resolved crystal structures, homology modeling was performed using their FASTA sequences via the SWISS-MODEL server (<https://swissmodel.expasy.org/>). Prior to docking, all heteroatoms and water molecules were removed from the protein structures to ensure accurate docking simulations, and the final structures were saved in.pdb format using Discovery Studio Visualizer.

2.2.1 AutoDock Vina

The prepared ligands in.pdbqt format were subjected to molecular docking against the identified target proteins using AutoDock Vina integrated within the PyRx platform (version 0.8; <https://pyrx.sourceforge.io/>) [31]. Further, the targets with which bioactive possessed the

least binding energy were visualized using Discovery Studio Visualizer.

2.2.2 Schrodinger suite glide

The LigPrep module of the Schrodinger suite (<https://www.schrodinger.com/products/ligprep>) was utilized to generate the 3D structures of the ligands. Protein structures were prepared using the Protein Preparation Wizard in Schrodinger Maestro (Schrodinger, LLC, New York, NY, 2021), wherein hydrogens were added, water molecules were removed, and protonation states were assigned. The OPLS 2005 force field was employed, and energy minimization was performed with a restraint of 0.3 Å RMSD to optimize the protein structure.

A receptor grid was generated based on the active site of the standard ligand to define the docking region. Subsequently, protein–ligand docking was performed using the glide module of Schrodinger Suite 2019 in extra precision (XP) mode, with the OPLS-3 force field implemented under flexible docking conditions to allow conformational variability of the ligands during binding. Upon completion, Glide scores (docking scores) were automatically calculated and protein–ligand interactions were completely analyzed with the help of the XP Visualizer tool in the glide module.

2.3 Molecular simulation and MMPBSA analysis

Protein–ligand complexes to be simulated through molecular dynamics (MD) were selected according to the lowest binding energies during molecular docking using the Schrodinger suite. These complexes are KRAS (PDB ID: 4OBE), TP53 (PDB ID: 1TUP), and IGF1R (PDB ID: 6PYH). The selected complexes are KRAS (PDB ID: 4OBE), TP53 (PDB ID: 1TUP), and IGF1R (PDB ID: 6PYH). MD simulations were performed using GROMACS version 2022.1 (<https://www.gromacs.org/>). Protein topologies were generated through the charmm36 forcefield using the pdb2gmx module, using appropriate force fields. Each protein–ligand complex was placed in a triclinic dodecahedron box, maintaining a minimum distance of 1 nm from the box edge, and solvated using the three-point transferable intermolecular potential (TIP3P) water model. To neutralize the system, sodium (Na⁺) and chloride (Cl[−]) ions were added as counterions. Energy minimization was carried out using the steepest descent algorithm, with the Verlet cutoff scheme, running for a maximum of 50,000 steps until a stable energy conformation was achieved. The minimized systems were then subjected to equilibration in two phases- NVT ensemble (constant number of particles, volume, and temperature) and NPT ensemble (constant number of particles, pressure, and temperature). The individual phases took 100

picoseconds as time, and temperature was controlled at 300 K on the V-rescale thermostat, and pressure was controlled at 1 bar in the C-rescale pressure coupling algorithm [29]. All protein–ligand complexes were initially simulated for 200 ns under identical conditions; however, the TP53-Hypoxiside A complex, which showed early equilibration and superior conformational stability, was further simulated up to 500 ns to confirm long-term trajectory convergence and binding robustness.

Particle Mesh Ewald (PME) method was used to compute long range electrostatic interactions accurately with both cutoff distance of 1.2 nm of Coulombic and van der Waals interaction. To maintain the integrity of bonded atoms during the simulation process bond constraints were managed using the LINCS algorithm. The production MD run of 200 ns was performed on each protein–ligand complex. The values of coordinates and energy were stored in 20 picoseconds during the simulation to be analyzed further. Analysis of the resultant trajectory files was conducted on the inbuilt GROMACS tools to obtain dynamic and structural information [32].

The gmx_MMPBSA module was used to compute the parameters of binding free energy and energy contribution [33]. To assess binding free energies and decompose the energy contributions, the gmx_MMPBSA module was employed. Calculations included parameters such as- Van der Waals and electrostatic interactions (molecular mechanics energies), Polar and non-polar solvation energies, non-polar solute–solvent interaction energies, total gas-phase and solvation energies, and overall binding free energies. A total of 100 representative frames were extracted from 10,000 simulation frames at intervals of 100 frames for MMPBSA calculations. The Poisson-Boltzmann Surface Area (PBSA) calculations were carried out using the internal PBSA solver within the Sander module. The resulting data were visualized and interpreted using the MMPBSA_ana module, providing comprehensive insights into the stability and strength of protein–ligand binding interactions.

3 Results

3.1 Network pharmacology

3.1.1 Mining of phytoconstituents

WFO showed 8 synonyms for *Solanum trilobatum* i.e., *Solanum acetosifolium*, *Solanum canaranum*, *Solanum fuscum*, *Solanum griffithii*, *Solanum hainanense* var. *griffithii*, *Solanum prostratum*, *Solanum trilobatum* var. *griffithii*, *Solanum trilobatum* var. *trilobatum*. The total number of bioactives was found to be 425 (Suppl. File 1 (sheet 1)).

3.1.2 Drug-likeness and ADMET profile, and biological spectra of bio-actives

The physiochemical properties of 425 bioactives was analysed (Suppl. File 1 (sheet 2)). Among these 205 scored a positive drug-likeness score with josamycin possessing the highest score of 1.46. Further, 65 bioactives showed zero violations to the Lipinski's rule of five. Biological spectrum analysis of all 425 bioactives further showed that 168 bioactives exhibit 'anti-neoplastic (breast cancer)' activity. From this set, compounds that satisfied both criteria- compliance with Lipinski's Rule of Five and anti-neoplastic (breast cancer) activity- were shortlisted.

The bioactive 22alpha,23alpha-epoxy-solanida-1,4,9-trien-3-one showed the highest blood–brain barrier permeability (0.9718), human intestinal absorption (1), highest affinity towards CYP450 3A4 substrate (0.8443), and biodegradation (0.9968). 5-hydroxy-7-methoxy-3-(3-methoxyphenyl)-8-methyl-4H-chromen-4-one showed highest Caco-2 permeability (0.9438). Hypoxiside A showed highest affinity to p-glycoprotein substrate (0.862), CYP450 3A4 inhibition (0.9496), and highest acute toxicity in rats (3.9586). Cytochalasin Z4 showed the highest p-glycoprotein (0.8249) and human ether-a-go-go-related gene inhibition (0.9152). 5,6-Epoxy-phomol showed highest affinity to renal organic cation transporter (0.9464) and CYP450 2C9 substrate (0.8918). 3a,16a-dihydroxyandrostenone showed highest affinity to CYP450 2D6 substrate (0.9097), and CYP450 2C9 inhibition (0.9369). 4-p-coumaroylquinic acid showed highest CYP450 1A2 Inhibition (0.9417), affinity to CYP inhibitory promiscuity (0.9725), AMES toxicity (0.9305) and acute oral toxicity (0.7858). Barogenin showed highest CYP450 2D6 inhibition (0.9363) and fish toxicity (1.3119). Rheochrysin with highest aqueous solubility (–2.2434) was found to be a strong carcinogen (0.9625). 4'-O-methylxanthohumol showed the highest honeybee toxicity (0.8623), and tetrahymena pyriformis toxicity (1.7841) (Fig. 1). 16 bioactives were selected for further investigation, as depicted in Fig. 2.

3.1.3 Identification of targets

A total of 2,141 breast cancer-related targets were identified, and 531 proteins were predicted to be modulated by the 16 bioactives. Among these, the common proteins were primarily associated with key cancer-related pathways, including microRNAs in cancer, PI3K-Akt signaling, endocrine resistance, HIF-1 signaling, focal adhesion, apoptosis, estrogen signaling, MAPK signaling, p53 signaling, hedgehog signaling, Wnt signaling, cAMP signaling, calcium signaling, mTOR signaling, VEGF signaling, choline metabolism in cancer, and adherens junction.

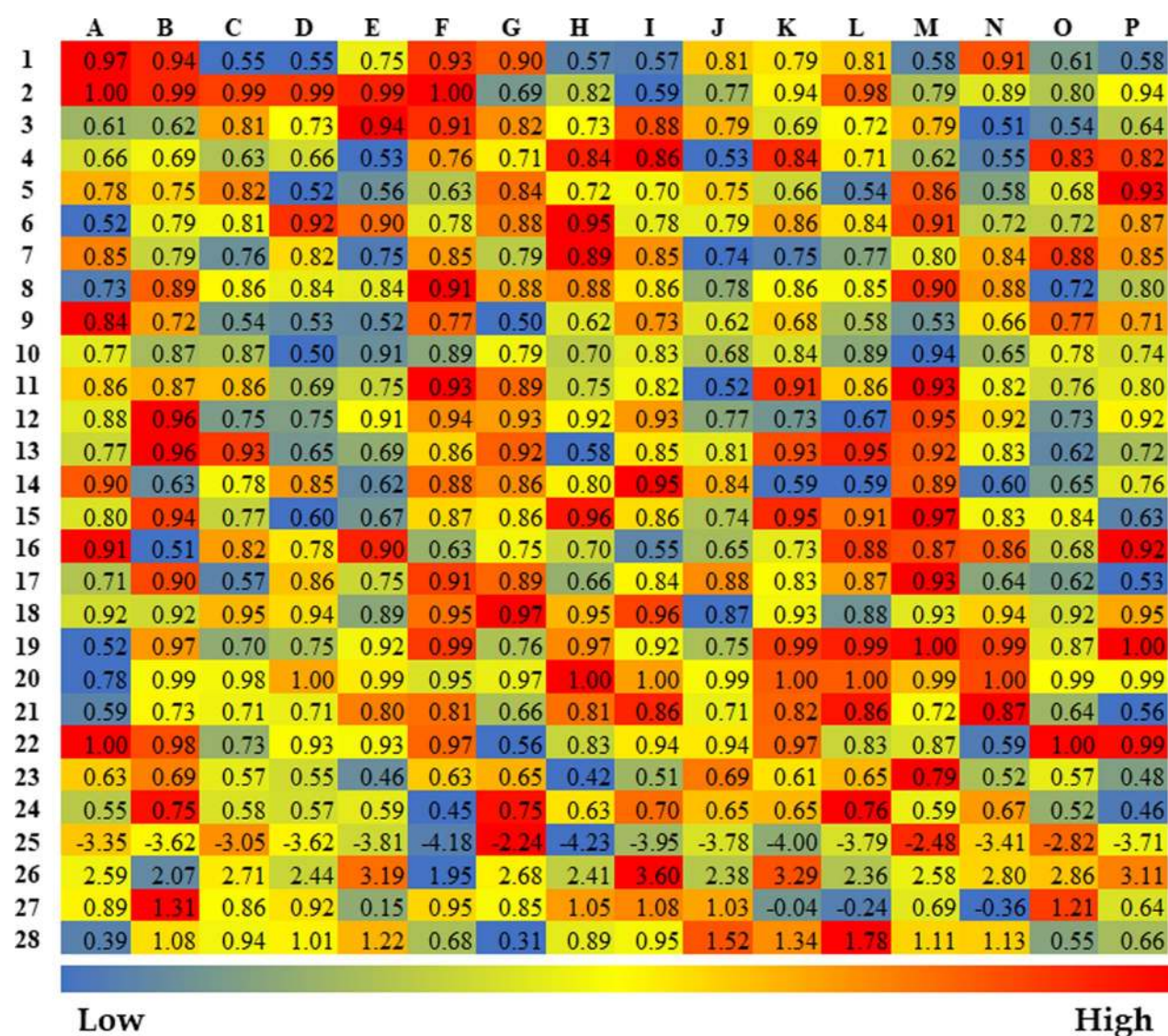


Fig. 1 ADMET profile of bio-actives via heat map. (1) Blood–Brain Barrier, (2) Human Intestinal Absorption, (3) Caco-2 Permeability, (4) P-glycoprotein Substrate, (5) P-glycoprotein Inhibitor, (6) Renal Organic Cation Transporter, (7) CYP450 2C9 Substrate, (8) CYP450 2D6 Substrate, (9) CYP450 3A4 Substrate, (10) CYP450 1A2 Inhibitor, (11) CYP450 2C9 Inhibitor, (12) CYP450 2D6 Inhibitor, (13) CYP450 2C19 Inhibitor, (14) CYP450 3A4 Inhibitor, (15) CYP Inhibitory Promiscuity, (16) Human Ether-a-go-go-Related Gene Inhibition, (17) AMES Toxicity, (18) Carcinogens, (19) Fish Toxicity, (20) Tetrahymena Pyriformis Toxicity, (21) Honey Bee Toxicity, (22) Biodegradation, (23) Acute Oral Toxicity, (24) Carcinogenicity (Three-class), (25) Aqueous solubility, (26) Rat Acute Toxicity, (27) Fish Toxicity, (28) Tetrahymena Pyriformis Toxicity of **A** 22alpha,23alpha-epoxy-so lanida-1,4,9-trien-3-one, **B** Barogenin, **C** 7,2'-dihydroxy-4' methoxyisoflavanol, **D** Catechin 7,4'-dimethyl ether, **E** 5-hydroxy-7-methoxy-3-(3-methoxyphenyl)-8-methyl-4H-chromen-4-one, **F** 3a,16a-Dihydroxyandrostene, **G** Rheochrysin, **H** 5,6-Epoxy-phomol, **I** Hypoxyside A, **J** griffithane B, **K** kwakhurin, **L** 4'-O-methylxanthohumol, **M** 4-p-coumaroylquinic acid, **N** 1-O-acetyl-4R,6S-britannilactone, **O** 7-methoxycrinamine Trifluoroacetic acid, **P** Cytochalasin Z4

3.1.4 Gene enrichment, gene ontology and network analysis
Protein–protein interaction (PPI) analysis was performed using the STRING database, and the resulting network was further enriched through KEGG pathway analysis, which identified 176 significant pathways. A protein–pathway interaction network was constructed using Cytoscape based on edge count, where the “PI3K-Akt

signaling pathway (*hsa04151*)” emerged as the most prominently modulated pathway (Table 1). Topological parameters further supported the prominence of this pathway. With an average shortest path length of 2.41, betweenness centrality of 0.045, and a closeness centrality of 0.41, it demonstrated substantial connectivity and central positioning within the network. Its high degree

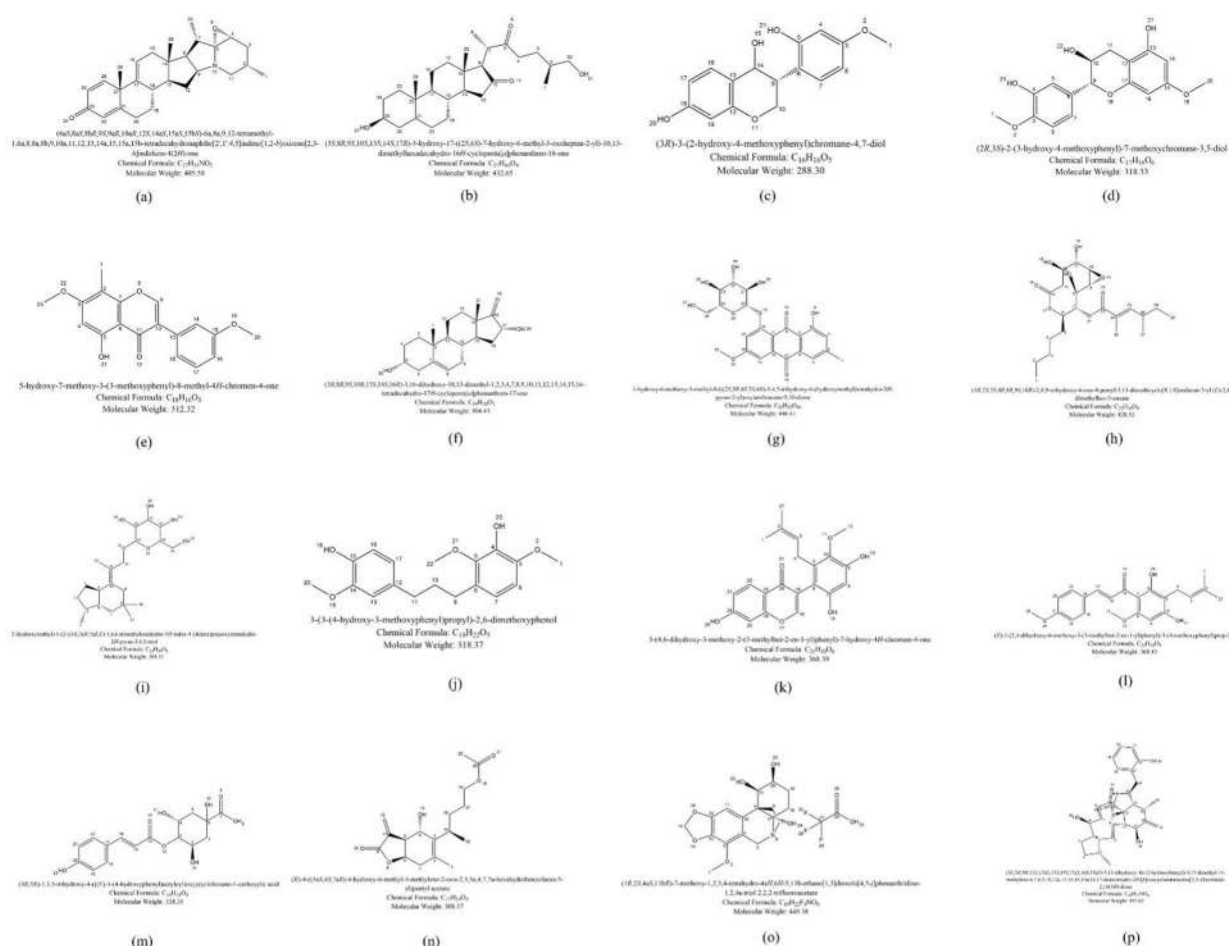


Table 1 Top 15 KEGG pathways modulated by *Solanum trilobatum* against breast cancer

Pathway (KEGG ID)	OGC/BGC	Strength	FDR	Genes
MicroRNAs in cancer (hsa05206)	33/159	1.43	2.20E−33	SIRT1, HMOX1, CCND1, CDKN1B, KRAS, PDGFRA, MDM2, CCNE1, PIK3CA, STAT3, CDK6, TP53, ERBB2, EGFR, ATM, MET, DNMT1, MTOR, PTGS2, PTEN, MMP9, ABL1, PLAU, PIM1, HDAC1, PIK3CD, BCL2, ABCC1, HRAS, CDKN2A, VIM, ABCB1, PIK3CB
PI3K-Akt signaling pathway (hsa04151)	39/349	1.16	1.10E−30	CCND1, CDKN1B, KRAS, PDGFRA, CDK4, MDM2, FLT4, CCNE1, KDR, PIK3CA, EGF, CDK6, CDK2, TP53, ERBB2, EGFR, FLT1, KIT, NOS3, INSR, MET, GSK3B, HSP90AA1, MTOR, PTEN, PIK3CD, JAK2, SPP1, BCL2, RELA, IL6, FGFR1, HRAS, FGFR2, PIK3CG, NTRK1, AKT1, IGF1R, PIK3CB
Endocrine resistance (hsa01522)	26/94	1.55	5.85E−29	MMP2, CCND1, CDKN1B, KRAS, CDK4, MDM2, PIK3CA, RB1, TP53, ERBB2, EGFR, ESR2, MTOR, JUN, NCOA3, MMP9, SRC, PIK3CD, GPER1, BCL2, ESR1, HRAS, CDKN2A, AKT1, IGF1R, PIK3CB
Breast cancer (hsa05224)	28/146	1.4	1.69E−27	CCND1, KRAS, CDK4, FLT4, PIK3CA, EGF, CDK6, RB1, TP53, ERBB2, EGFR, KIT, GSK3B, PGR, ESR2, MTOR, JUN, PTEN, NCOA3, PIK3CD, BRCA2, FGFR1, ESR1, HRAS, AKT1, CTNNB1, IGF1R, PIK3CB
HIF-1 signaling pathway (hsa04066)	23/102	1.47	5.64E−24	HMOX1, CDKN1B, PIK3CA, STAT3, EGF, ERBB2, EGFR, FLT1, NOS3, INSR, NOS2, MTOR, PIK3CD, TFRC, BCL2, RELA, IL6, SLC2A1, HIF1A, AKT1, ENO1, IGF1R, PIK3CB
Focal adhesion (hsa04510)	23/195	1.18	8.26E−19	CCND1, PDGFRA, FLT4, KDR, PIK3CA, EGF, ERBB2, EGFR, FLT1, MET, GSK3B, JUN, PTEN, SRC, PIK3CD, SPP1, BCL2, HRAS, AKT1, BIRC2, CTNNB1, IGF1R, PIK3CB
Apoptosis (hsa04210)	19/131	1.27	4.37E−17	NFKBIA, KRAS, PIK3CA, TP53, ATM, CASP8, PARP1, CASP7, JUN, PIK3CD, BCL2, RELA, TNF, HRAS, NTRK1, DDIT3, AKT1, BIRC2, PIK3CB
Estrogen signaling pathway (hsa04915)	19/133	1.27	5.49E−17	MMP2, KRAS, PIK3CA, EGFR, NOS3, PGR, HSP90AA1, ESR2, JUN, NCOA3, MMP9, SRC, PIK3CD, GPER1, BCL2, ESR1, HRAS, AKT1, PIK3CB
Prolactin signaling pathway (hsa04917)	15/68	1.46	5.62E−16	CCND1, KRAS, PIK3CA, STAT3, GSK3B, ESR2, CYP17A1, SRC, PIK3CD, JAK2, RELA, ESR1, HRAS, AKT1, PIK3CB
MAPK signaling pathway (hsa04010)	23/286	1.02	1.52E−15	KRAS, PDGFRA, FLT4, IL1B, KDR, EGF, TP53, ERBB2, EGFR, FLT1, KIT, INSR, MET, JUN, RELA, FGFR1, TNF, HRAS, FGFR2, NTRK1, DDIT3, AKT1, IGF1R
Cell cycle (hsa04110)	17/120	1.26	3.08E−15	CCND1, CDKN1B, CDK7, CDK4, MDM2, CCNE1, CDK6, CDK2, RB1, TP53, ATM, GSK3B, ABL1, HDAC1, CDK1, CHEK1, CDKN2A
ErbB signaling pathway (hsa04012)	15/81	1.38	4.96E−15	CDKN1B, KRAS, PIK3CA, EGF, ERBB2, EGFR, GSK3B, MTOR, JUN, ABL1, SRC, PIK3CD, HRAS, AKT1, PIK3CB
p53 signaling pathway (hsa04115)	14/72	1.4	2.37E−14	CCND1, CDK4, MDM2, CCNE1, CDK6, CDK2, TP53, ATM, CASP8, PTEN, CDK1, BCL2, CHEK1, CDKN2A
Adherens junction (hsa04520)	11/69	1.32	1.13E−10	SNAI2, SNAI1, ERBB2, EGFR, INSR, MET, PTPN1, SRC, FGFR1, CTNNB1, IGF1R
Choline metabolism in cancer (hsa05231)	12/95	1.21	1.56E−10	KRAS, PDGFRA, PIK3CA, EGF, EGFR, MTOR, JUN, PIK3CD, HRAS, HIF1A, AKT1, PIK3CB
VEGF signaling pathway (hsa04370)	10/56	1.36	3.24E−10	KRAS, KDR, PIK3CA, NOS3, PTGS2, SRC, PIK3CD, HRAS, AKT1, PIK3CB
mTOR signaling pathway (hsa04150)	12/150	1.02	1.61E−08	KRAS, PIK3CA, INSR, GSK3B, MTOR, PTEN, PIK3CD, TNF, HRAS, AKT1, IGF1R, PIK3CB
Cytokine-cytokine receptor interaction (hsa04060)	11/282	0.7	4.43E−05	IL12B, IL1B, CXCL8, CD40, BMP2, CXCL12, IL6, CXCR4, TNF, IL10, CXCL2
Calcium signaling pathway (hsa04020)	8/191	0.73	0.00038	PDGFRA, EGF, ERBB2, EGFR, NOS3, ADRB2, NOS2, CXCR4
cAMP signaling pathway (hsa04024)	8/207	0.7	0.00063	NFKBIA, PIK3CA, ADRB2, JUN, PIK3CD, RELA, AKT1, PIK3CB
Wnt signaling pathway (hsa04310)	6/154	0.7	0.0034	CCND1, TP53, GSK3B, JUN, DKK1, CTNNB1
Hedgehog signaling pathway (hsa04340)	3/46	0.93	0.0131	CCND1, GSK3B, BCL2

FDR: False Discovery Rate; OGC: Observed Gene Count; BGC: Background Gene Count

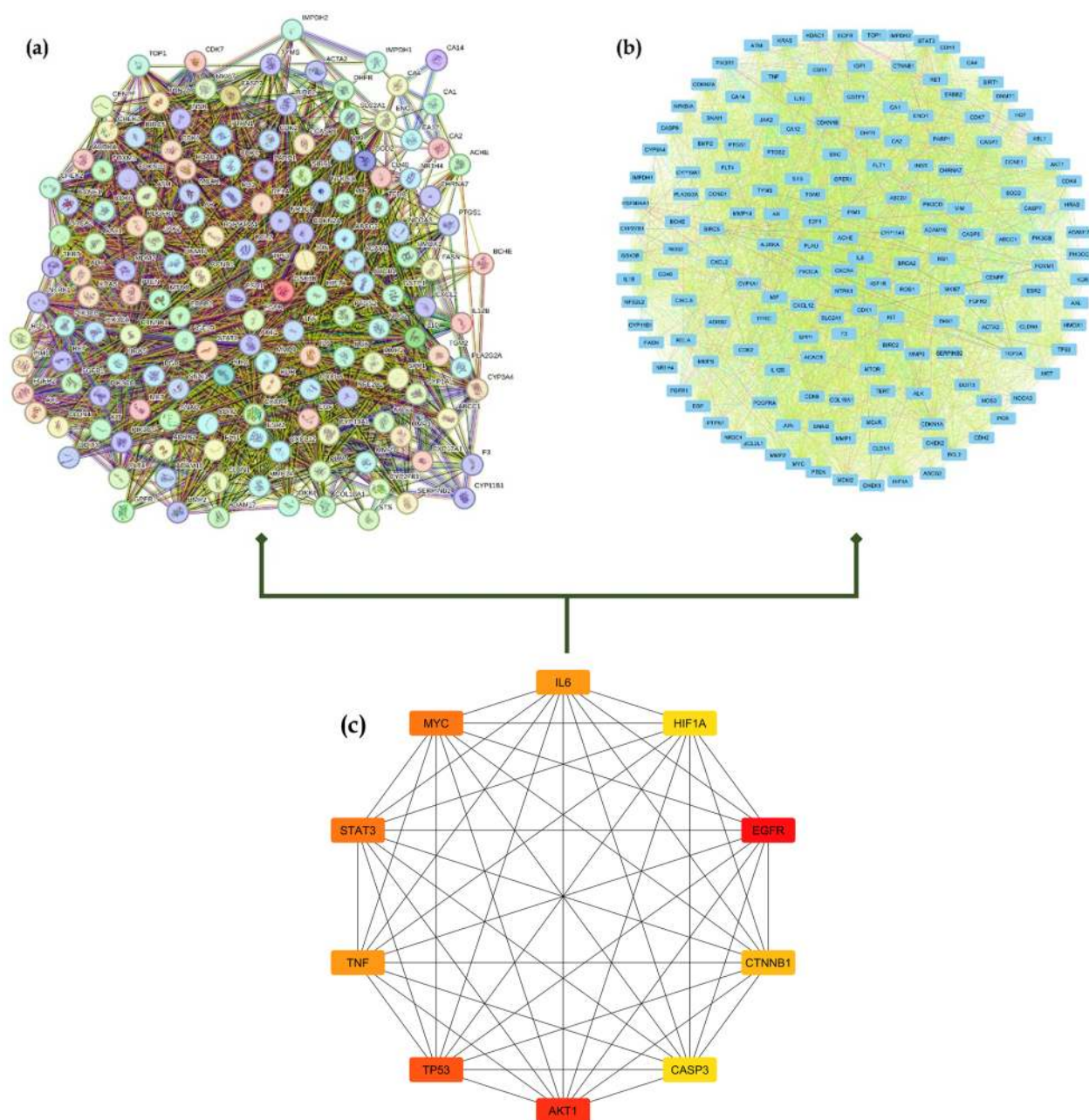


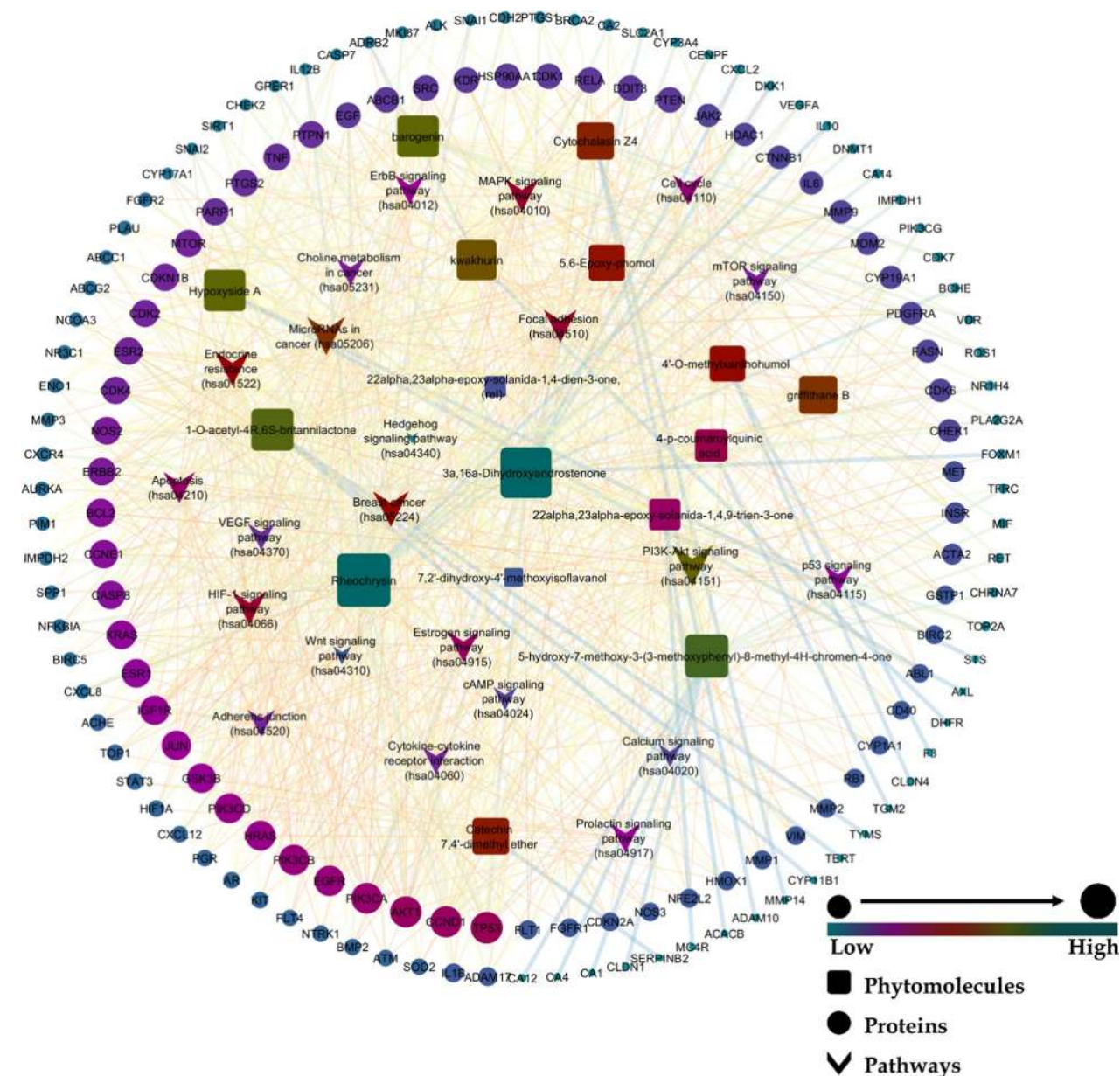
Fig. 3 Protein-protein interaction of targets modulated by bioactives in *S. trilobatum* against breast cancer cells represented via **a** STRING, **b** Cytoscape, **c** Hub genes

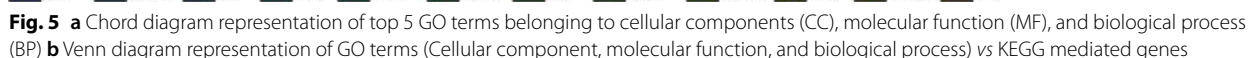
RET, *ROS1*, *CD40*, *BMP2*, *JAK2*, *ALK*, *TFRC*, *IL6*, *FGFR1*, *CHRNA7*, *FGFR2*, *NTRK1*, *NR1H4*, *BIRC2*, *IGF1R* against 418 background genes at a strength of 0.92. Similarly, 156 MF were identified in which protein binding (GO:0005515) scored the lowest false discovery rate of 1.84×10^{-28} via the modulation of 127 observed genes *i.e.* *SIRT1*, *MIF*, *HMOX1*, *NFKBIA*, *AURKA*, *MMP2*, *ACTA2*, *CCND1*, *CDKN1B*, *IL12B*, *NR3C1*, *SNAIL1*, *KRAS*, *CDK7*, *PDGFRA*, *CDK4*,

MDM2, *ADAM10*, *FLT4*, *CCNE1*, *IL1B*, *KDR*, *PIK3CA*, *BCHE*, *STAT3*, *EGE*, *CDK6*, *CDK2*, *RB1*, *CDH2*, *TP53*, *ERBB2*, *EGFR*, *ATM*, *FLT1*, *KIT*, *CLDN1*, *NOS3*, *MC4R*, *AXL*, *BIRC5*, *ACHE*, *INSR*, *FASN*, *ADRB2*, *CXCL8*, *MMP14*, *TERT*, *ADAM17*, *TYMS*, *MET*, *IMPDH2*, *GSK3B*, *PGR*, *NOS2*, *F3*, *HSP90AA1*, *ACACB*, *FOXM1*, *ESR2*, *IMPDH1*, *CASP8*, *COL18A1*, *TOP1*, *MTOR*, *PARP1*, *CENPE*, *PTGS2*, *ROS1*, *MKI67*, *JUN*, *PTPN1*, *PTEN*, *NCOA3*, *CD40*, *MMP9*, *ABL1*, *PIM1*, *HDAC1*,

SRC, DKK1, AR, BMP2, BRCA2, JAK2, CHEK2, ALK, TFRC, CYP1A1, SPP1, CDK1, CXCL12, NFE2L2, BCL2, GSTP1, RELA, IL6, CXCR4, CHEK1, FGFR1, TNF, ESR1, CHRNA7, HRAS, CLDN4, FGFR2, TOP2A, IL10, SLC2A1, CDKN2A, PIK3CG, CXCL2, NTRK1, HIF1A, VIM, SOD2, NR1H4, DDIT3, AKT1, BIRC2, ABCB1, CTNNB1, ENO1, IGF1R, ABCG2, CYP3A4, PIK3CB against 7242 background genes at a strength of 0.36. Moreover, 1942 BP were identified where, response to chemical (GO:0010033) scored the lowest false discovery rate of 1.45E-54 via the modulation

of 109 observed genes i.e. *SNAI2, SIRT1, HMOX1, NFKBIA, MMP2, CCND1, CYP27B1, CDKN1B, IL12B, NR3C1, PDGFRA, CDK4, MDM2, ADAM10, FLT4, IL1B, KDR, PIK3CA, BCHE, STAT3, RB1, TP53, ERBB2, EGFR, ATM, FLT1, CA2, KIT, CYP11B1, CLDN1, NOS3, MC4R, MMP3, AXL, ACHE, INSR, FASN, ADRB2, CXCL8, MMP14, ADAM17, TYMS, IMPDH2, GSK3B, PGR, NOS2, F3, HSP90AA1, ACACB, ESR2, RET, CASP8, DNMT1, MTOR, TGM2, PARP1, PTGS2, CASP7, JUN, PTPN1, NCOA3, CD40, MMP9, ABL1, PIM1, HDAC1, SRC, DKK1, AR, PIK3CD,*





Molecular docking via AutoDock vina revealed *HRAS*, *KRAS*, *NCOA3*, *PGR*, and *PIK3CB* possess the least binding energy of -9.4 , -9.1 , -9 , -9.8 , and -9.6 kcal/mol with ligands rheochrysin, hypoxiside, cytochalasin, rheochrysin, and 4-p-coumaroylquinic acid respectively (**Suppl. File 4**). Rheochrysin has a polyphenolic backbone with multiple hydroxyl groups ($-\text{OH}$) and conjugated aromatic rings. The docking interaction revealed six hydrogen bonds with amino acids *GLY13*, *GLU31*, *LYS117*, *THR58*, and *THR35*. Hypoxiside, forms seven hydrogen bonds with key amino acids, including *ASP30*, *TYR32*, *SER17*, *LYS16*, *VAL14*, and *GLY15* and all are facilitated by its multiple hydroxyl ($-\text{OH}$) groups rather than extensive pi-pi stacking. Additionally, hypoxiside also engages in four pi interactions involving aromatic residues *LYS117*, *PHE28*, *LYS147*, and *LEU120*, which is through localized pi interactions. Cytochalasin,

a fungal-derived macrocyclic alkaloid, is likely to be present in *S. trilobatum* because endophytic fungi closely associated with plants has hydroxyl (–OH), amide (–CONH), and aromatic structure, which play a crucial role in its binding affinity. The docking showed it forms three hydrogen bonds with the amino acids *GLU681*, *PRO682*, and *TRP751*, mediated by its hydroxyl and amide groups enhancing its stability within the *NCOA3* binding pocket. The docking analysis of rheochrysin revealed it forms seven hydrogen bonds with the amino acids *PHE778*, *ILE699*, *ARG766*, *MET759*, *GLN725*, and *GLN815*. These interactions are mediated by its hydroxyl (–OH) groups, which act as hydrogen bond donors, stabilizing rheochrysin within the receptor's binding pocket. The docking analysis of 4-p-Coumaroylquinic acid, a phenolic ester, structurally having a quinic acid core linked to p-coumaric acid moiety, revealed two hydrogen bonds with *TRP381* and *LYS342*, formed via its hydroxyl (–OH) and carboxyl (–COOH) groups. Figure 6 represents the binding energy as obtained from AutoDock vina and Fig. 7 represents 3D and 2D interaction these compounds.

Docking in glide displayed *KRAS*, *IGF1R* and *TP53* to possess the least docking score of –9.71, –7.9, and –7.71 kcal/mol with 4-p-coumaroylquinic acid, kwakhurin and hypoxyside A respectively (Suppl. File 5). The docking analysis of 4-p-coumaroylquinic acid with *KRAS* revealed six hydrogen bonds with *ASP119*, *TYR32*, *GLY13*, *SER17*, and *LYS16*, mediated by its hydroxyl (–OH) groups. Additionally, two pi interactions with *LYS117* and *PHE28* suggest the involvement of hydrophobic and aromatic stacking interactions, further reinforcing its binding affinity. Kwakhurin, a prenylated

isoflavonoid, with a binding energy of –7.9 kcal/mol exhibited notable binding interactions with *IGF1R* when docking in glide. Structurally, Kwakhurin is an isoflavonoid core with a prenyl substitution, which enhances hydrophobic interactions and binding affinity. The docking analysis revealed four hydrogen bonds with *THR1127*, *MET1126*, *ASP1123*, and *GLY1122* mediated by its hydroxyl (–OH) groups. Docking analysis of hypoxyside A, showed it had 2 hydrogen bonds with *SER269* and *LEU111*, which was mediated by the hydroxyl (–OH) group. The binding energy of glide was obtained, and the data is represented in Fig. 8 and the interaction of these compounds in 3D and 2D represented in Fig. 9.

3.3 Molecular stability of docked complexes

The molecular dynamics (MD) simulation of *KRAS* -4-p-coumaroylquinic acid complex, *IGF1R* kwakhurin complex, and *TP53* Hypoxyside A complex in 200ns, revealed the structural dynamics, stability, and behaviour of the complex in the environment of breast cancer. Root mean square deviation(RMSD), root mean square fluctuation(RMSF), radius of gyration(Rg), solvent-accessible surface area(SASA), and hydrogen bonding were determined as vital parameters to meet the effect of the ligand on the dynamics of the proteins [34]. In comparison to other complexes, in case of *TP53*-Hypoxyside A complex the RMSD stabilized only after about 200 ns and therefore required a long period of simulation until 500 ns.

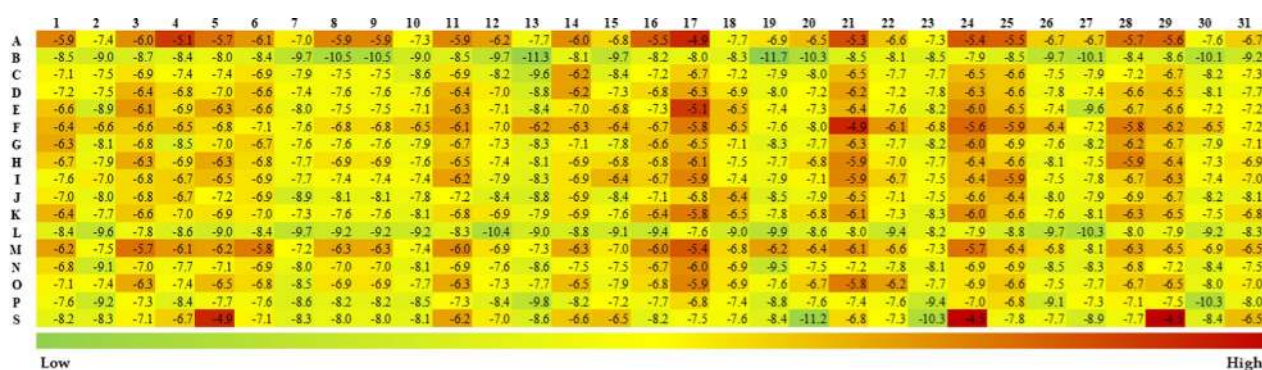


Fig. 6 Binding energy of **A** 22alpha,23alpha-epoxy-solanida-1,4,9-trien-3-one, **B** Barorgenin, **C** 7,2'-dihydroxy-4'-methoxyisoflavanol, **D** Catechin 7,4'-dimethyl ether, **E** 1-O-acetyl-4R,6S-britannilactone **F** 3a,16a-Dihydroxyandrosteneone **G** 4'-O-methylxanthohumol, **H** 4-p-coumaroylquinic acid, **I** 5,6-Epoxy-phomol, **J** 5-hydroxy-7-methoxy-3-(3-methoxyphenyl)-8-methyl-4H-chromen-4-one, **K** 7-methoxycrinamine Trifluoroacetic acid, **L** Cytochalasin Z4, **M** griffithane B, **N** Hypoxyside A, **O** kwakhurin, **P** Rheochrysin, **S** Standard* with targets (1) *CCND1*, (2) *KRAS*, (3) *CDK4*, (4) *FLT4*, (5) *PIK3CA*, (6) *EGF*, (7) *CDK6*, (8) *RB1*, (9) *ERBB2*, (10) *EGFR*, (11) *KIT*, (12) *GSK3B*, (13) *PGR*, (14) *ESR2*, (15) *MTOR*, (16) *JUN*, (17) *PTEN*, (18) *NCOA3*, (19) *PIK3CD*, (20) *BRCA2*, (21) *FGFR1*, (22) *ESR1*, (23) *HRAS*, (24) *AKT1*, (25) *CTNNB1*, (26) *IGF1R*, (27) *PIK3CB*, (28) *CASP8*, (29) *TP53*, (30) *NOS2*, (31) *PTPN1* when docked in Autodock vina (kcal/mol). *Standard for respective target

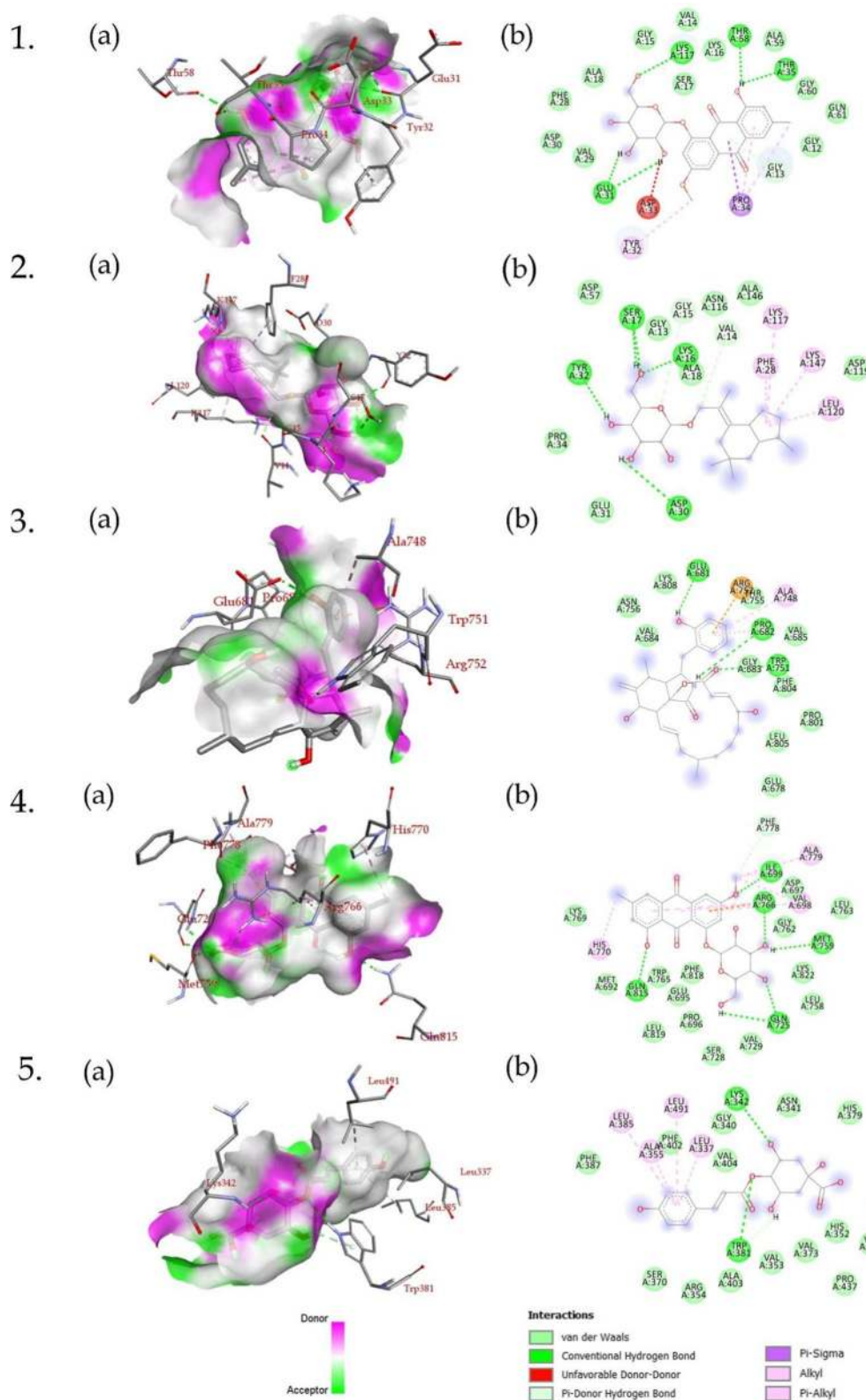


Fig. 7 a 3D and b 2D interaction of (1) *HRAS* with rheochrysin, (2) *KRAS* with hypoxiside, (3) *NCOA3* with cytochalasin, (4) *PGR* with rheochrysin, (5) *PIK3CB* with 4-p-coumaroylquinic acid when docked in AutoDock Vina

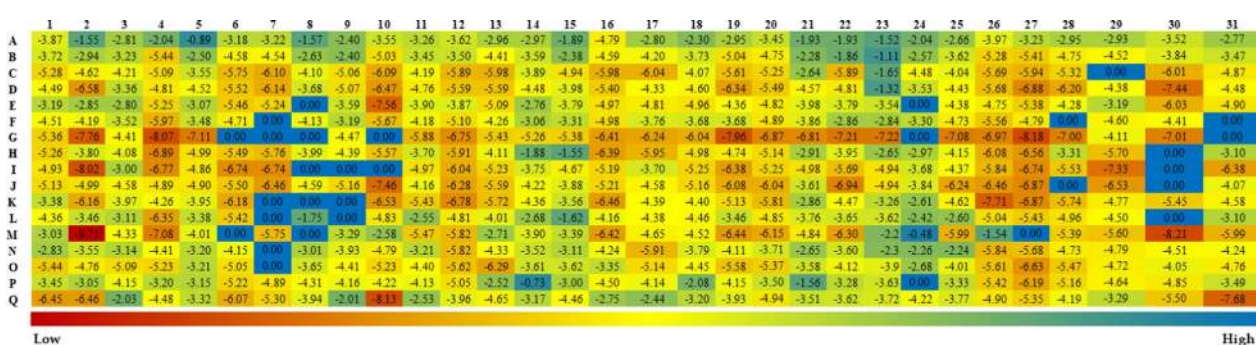


Fig. 8 Binding energy of **A** 22alpha,23alpha-epoxy-solanida-1,4,9-trien-3-one, **B** Barogenin, **C** 7,2'-dihydroxy-4'-methoxyisoflavanol, **D** Catechin, **E** 7,4'-dimethyl ether, **F** 5-hydroxy-7-methoxy-3-(3-methoxyphenyl)-8-methyl-4H-chromen-4-one, **G** 3a,16a-Dihydroxyandrostene, **H** Rheochrysin, **I** 5,6-Epoxy-chloro, **J** Hypoxyside A, **K** griffithane B, **L** 4'-O-methylxanthohumol, **M** 4-p-coumaroylquinic acid, **N** 1-O-acetyl-4R,6S-br itannilactone, **O** 7-methoxycinnamabine Trifluoroacetic acid, **P** Cytochalasin Z4, **S** Standard with targets (1) CCND1, (2) KRAS, (3) CDK4, (4) FLT4, (5) PIK3CA, (6) EGF, (7) CDK6, (8) RB1, (9) ERBB2, (10) EGFR, (11) KIT, (12) GSK3B, (13) PGR, (14) ESR2, (15) MTOR, (16) JUN, (17) PTEN, (18) NCOA3, (19) PIK3CD, (20) BRCA2, (21) FGFR1, (22) ESR1, (23) HRAS, (24) AKT1, (25) CTNNB1, (26) IGF1R, (27) PIK3CB, (28) CASP8, (29) TP53, (30) NOS2, (31) PTPN1 when docked in Schrodinger suite Glide

3.3.1 KRAS 4-p-coumaroylquinic acid complex

The backbone RMSD indicated that initially, up to a point of 51 ns, the deviations had a range of 0.9–2.0 Å, after which, a relatively stable state was recorded until 114 ns. A sharp rise was observed with a peak at 115.4 ns then a decrease at 128.2 ns and an upward trend at the end of the simulation. Simultaneously the complex was characterized by a somewhat broader range of fluctuation (1.4–2.8 Å), and after 52.2 ns till 115.2 ns. It also had a maximum of 115.4 ns and dip of 123.6 ns. It is important to mention that a steady gap of about 1 Å between complex and backbone RMSDs was seen all through the simulation. The fluctuation of Ca atoms of the protein in RMSF analysis was between about 0.4–5 Å. The ligand had marginally higher RMSF values with a range of about 0.5–6 Å to mean that portions of the ligand remained with a certain amount of mobility in the binding site. This does not imply instability, but gives an indication of an adaptive binding mode, the ligand changes its conformation to fit excellently into the dynamic binding pocket of the protein. Rg values of KRAS protein were relatively stable over the molecular dynamic path of 200 ns with a few reversal changes observed between the range of 10–15 Å. Such fluctuation is not out of scale of a globular protein, and it was observed to mean that the protein maintained compact and folded form throughout the simulation. The SASA remained more or less constant as it ranged between 90 and 100 nm² during the 200 ns simulation time. The highest number of hydrogen bonds between KRAS and 4-p-coumaroylquinic acid obtained was six as observed over a period of 200 ns. Nevertheless, these were not constant and long-term interactions. A total energy decomposition analysis was conducted to obtain a better understanding of the binding energetics

and the amino acid residues that lead to the stability of complex. This discussion showed that some residues—namely GLY15, PHE28, GLU31, PRO34, and LYS147—were important in the dynamics of the interaction. It is also notable that PHE28 had the highest binding energy (−1.11 kcal/mol), which indicates that there is a strong interaction between the ligand and this enzyme. The moderate energy contributions of LYS147 (−0.80 kcal/mol), GLY15 (−0.64 kcal/mol) and GLU31 (−0.40 kcal/mol) also indicate stability. In contrast, LYS117 showed a positive energy contribution (+1.86 kcal/mol), indicating destabilizing interaction (Fig. 10, Movie 1). This might be as a result of electrostatic repulsion, solvent exposure or strain that occurs to the ligand during binding.

3.3.2 IGF1R—Kwakhurin complex

RMSD analysis of the protein backbone, and of the ligand-bound complex showed that there were an early-stage conformational accommodation and a general increase in structural stability as a whole. Peaks were seen at ~3.0 Å at 5.4 ns, and ~4.2 Å at 3.2 ns with the backbone RMSD and the complex, respectively. These show initial relaxation of the system and orientation of the ligand in the binding site. The following drops at 63.8 ns to 71.8 ns (2.1–2.3 Å for backbone) and from 113.2 to 120.6 ns (2.7–2.8 Å) are apparently short-lived conformational changes. The peak of the backbone was 109.2 ns (~4.0 Å), are apparently short-lived conformational changes. The peak of the backbone was (~4.5 Å). which was the maximum of the complex. With this fluctuation, both tracks however became stable in the latter half of the simulation. The observed difference in the RMSD of the backbone and complex of magnitude of ~0.5 Å likely indicates average flexibilities

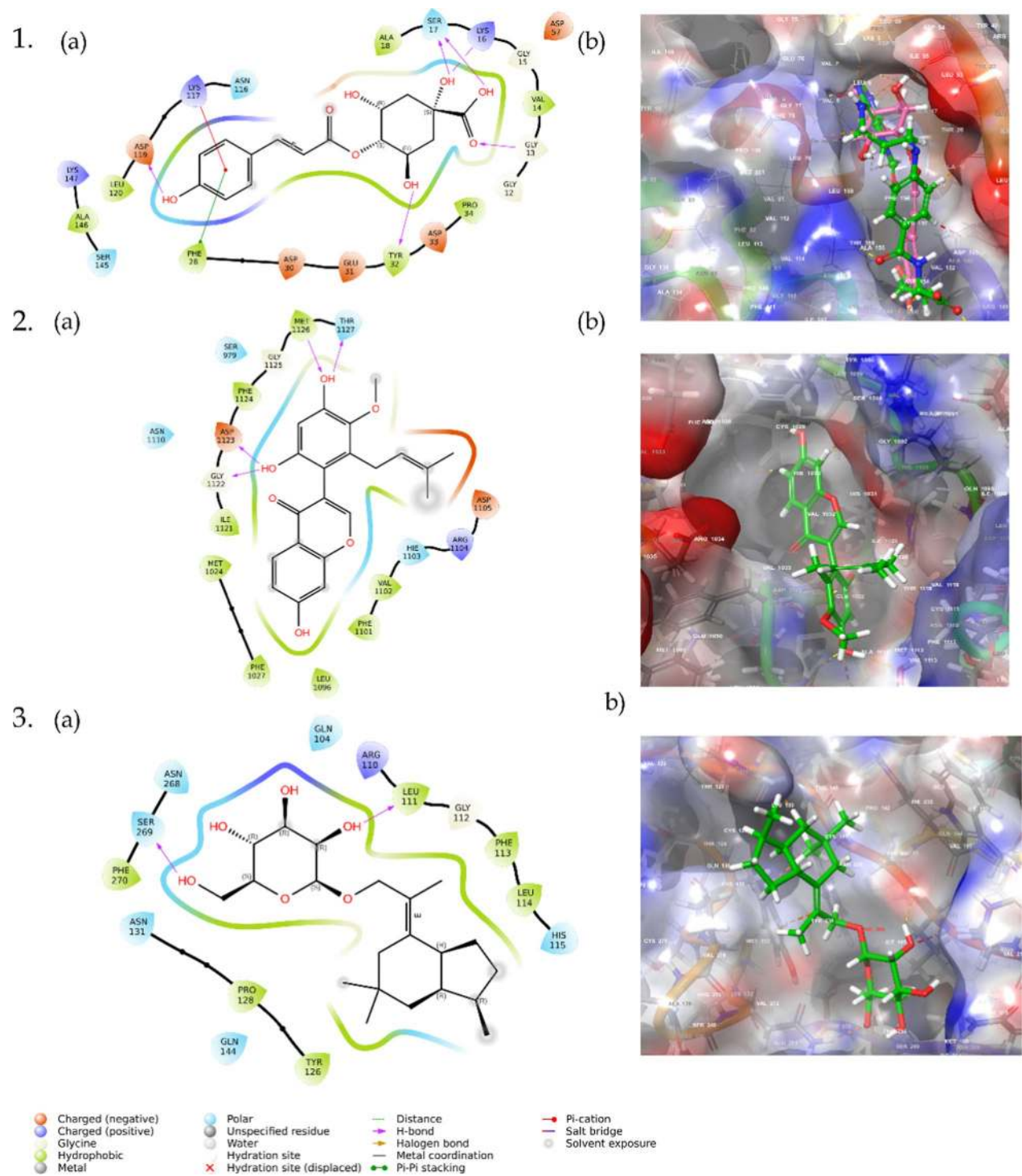


Fig. 9 a 2D and b 3D interaction of (1) *KRAS* with 4-p-coumaroylquinic acid when docked in Schrodinger suite Glide, (2) *IGF1R* with Kwakhurin when docked in Schrodinger suite Glide, (3) *TP53* with Hypoxiside A when docked in Schrodinger suite Glide

in the structure with no destabilization of the protein by the ligand. RMSF values of the C α atoms were found to be within the range of ~0.4 to ~9.0 Å and that of the ligand varied between ~0.5 to ~9.4 Å, the major variation (1.0–1.5 Å). The effect of this consistency is that the overall compactness of the IGF1R protein was

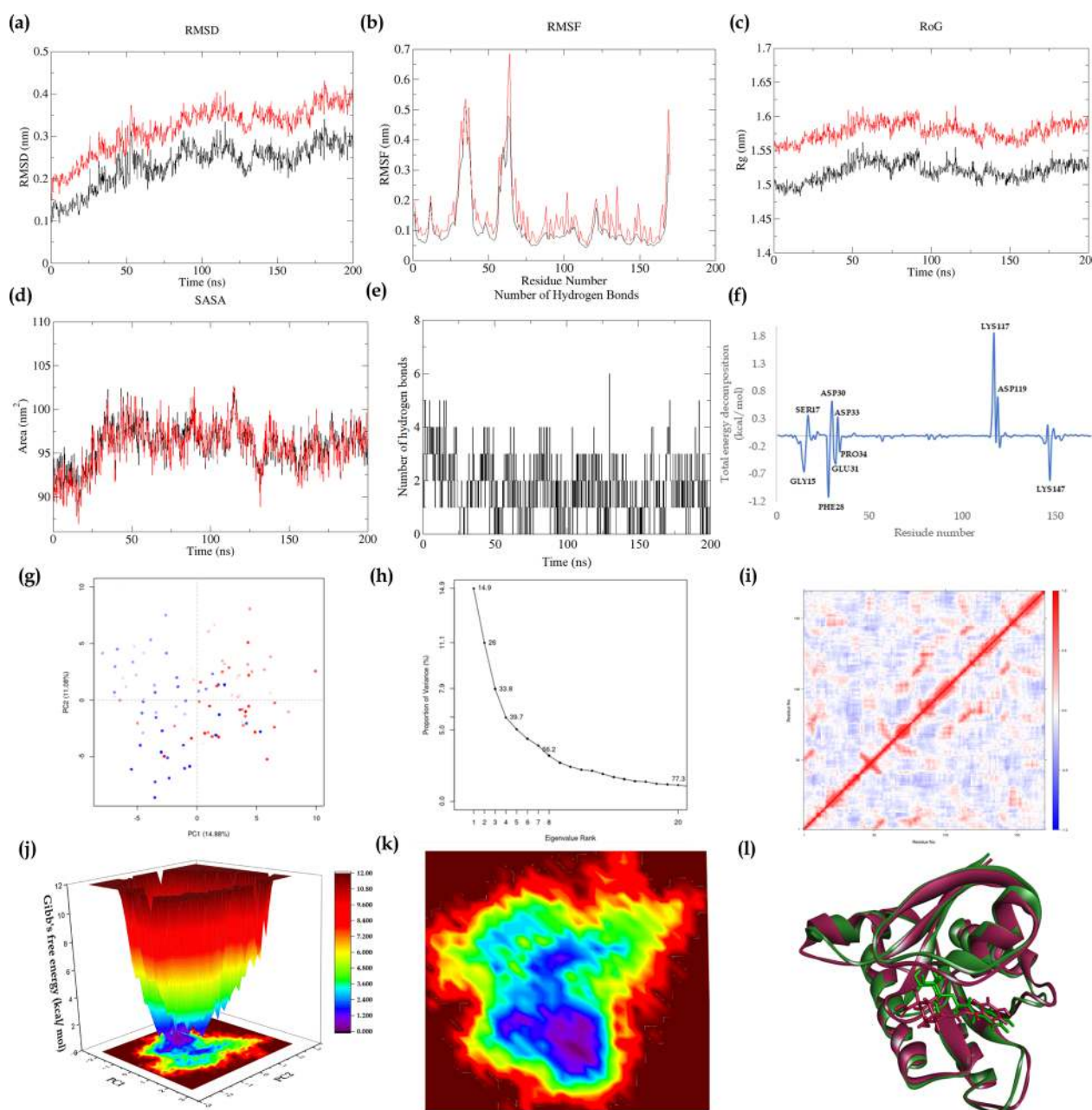


Fig. 10 Parameters describing the stability of complex KRAS- 4-p-coumaroylquinic acid **a** RMSD of backbone (black) and complex (red) **b** RMSF for c-alpha atoms (black) and complex (red) **c** RoG of backbone (black) and complex (red) **d** SASA of protein (black) and complex (red) **e** Number of hydrogen bonds formed **f** Total energy decomposition per residue **g, h** Principal component analysis (PCA) of motion projection plot for amino acid C- α atoms in phase space along the PC1 and PC2 and eigenvector rank plot using first 20 eigenvectors value. The continuous color spectrum of dots from blue to white to red signifies the MD simulation time scale (0–200 ns). The initial, intermediary, and finishing timescales are represented by blue, white, and red, respectively **i** Dynamic cross- correlation matrix (DCCM) plots for amino acid residues present in the three complex dynamic systems **j** 3D plot free energy landscape projection using PC1 and PC2 **k** 2D plot of free energy landscape projection using PC1 and PC2 **l** 3D interaction of complex with ligand depicted as green (start of simulation) and blue (end of simulation) and surface on the 3D interaction with respect to hydrogen bond donors and acceptors. Where, different colors on protein represent different residues

maintained during the simulation. The similarity in the Rg profiles of the free and bound forms an additional argument in favor of structural integrity of the protein under the both the conditions, which is to prove that the

binding of kwakhurin does not trigger domain collapse or unfolding. The protein values at the level of SASA were between 157 and 177 nm² and the complex had values between 155 and 175 nm² and no significant deviation

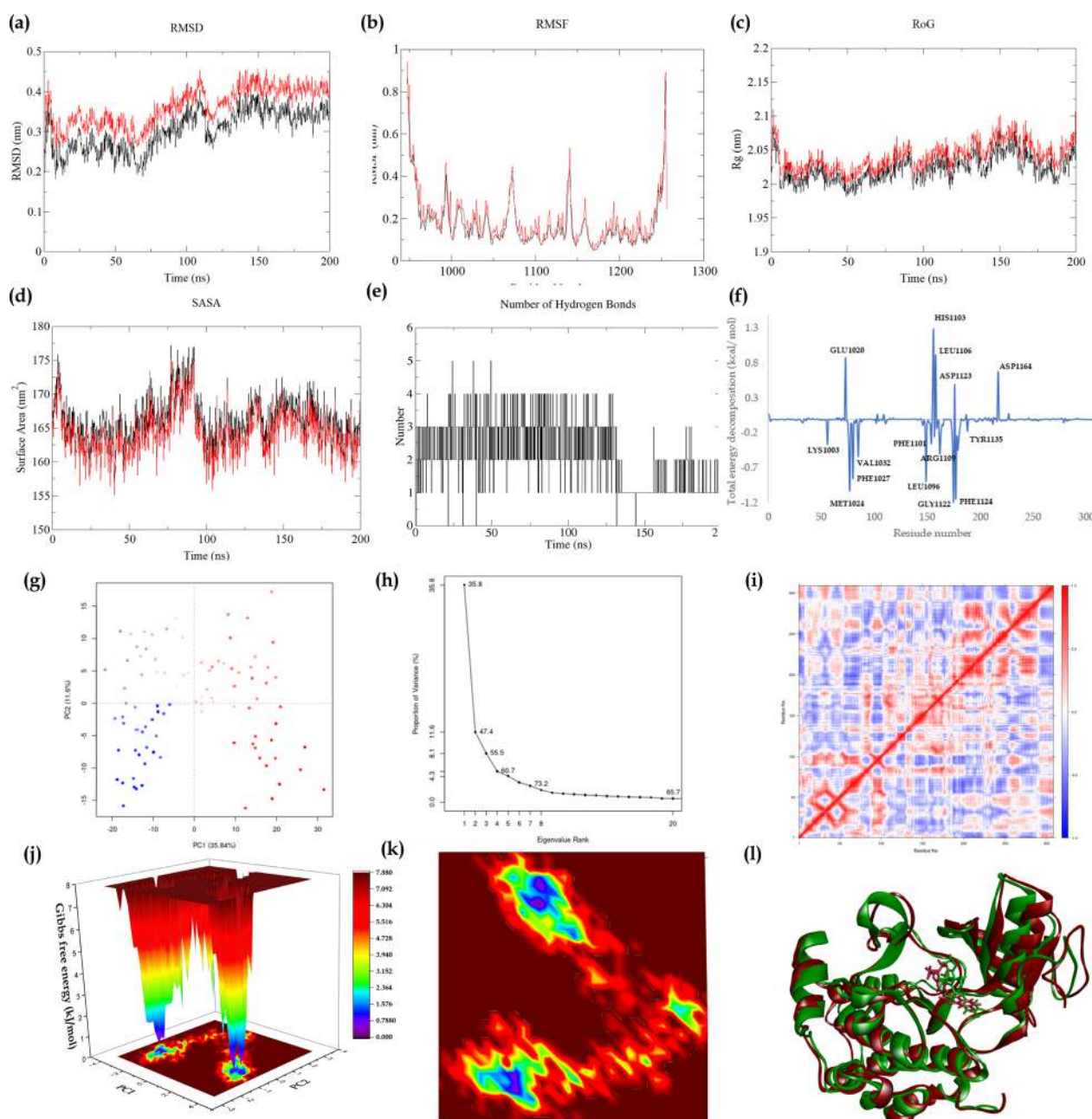


Fig. 11 Parameters describing the stability of complex *IGF1R*- Kwakhurin complex **a** RMSD of backbone (black) and complex (red) **b** RMSF for C- α atoms (black) and complex (red) **c** RoG of backbone (black) and complex (red) **d** SASA of protein (black) and complex (red) **e** Number of hydrogen bonds formed **f** Total energy decomposition per residue **g, h** Principal component analysis (PCA) of motion projection plot for amino acid C- α atoms in phase space along the PC1 and PC2 and eigenvector rank plot using first 20 eigenvectors value. The continuous color spectrum of dots from blue to white to red signifies the MD simulation time scale (0–200 ns). The initial, intermediary, and finishing timescales are represented by blue, white, and red, respectively **i** Dynamic cross-correlation matrix (DCCM) plots for amino acid residues present in the three complex dynamic systems **j** 3D plot free energy landscape projection using PC1 and PC2 **k** 2D plot of free energy landscape projection using PC1 and PC2 **l** 3D interaction of complex with ligand depicted as green (start of simulation) and blue (end of simulation) and surface on the 3D interaction with respect to hydrogen bond donors and acceptors. Where, different colors on protein represent different residues

was recorded during the simulation. The steady SASA indicates no major conformational transitions such as domain expansion or closure occurred. A maximum of

five hydrogen bonds were observed between the ligand and protein during the trajectory, and four persistent hydrogen bond interactions were maintained almost

throughout. The per-residue energy decomposition analysis for the *IGF1R*- Kwakhurin complex provides critical insights into the local binding environment and the nature of protein- ligand interactions at an atomic level. Among the residues analysed, *PHE1124* (−1.13 kcal/mol) exhibited the most favourable contribution. Other notable contributors included *MET1024* (−1.02 kcal/mol), *LEU1096* (−0.89 kcal/mol), and *PHE1027* (−0.85 kcal/mol). On the other hand, *HIS1103* contributed unfavourably (+1.29 kcal/mol) to the total interaction energy (Fig. 11, Movie 2). This may reflect electrostatic repulsion, unfavourable desolvation effects, or a steric clash that creates local strain upon ligand accommodation. However, the entire energetic landscape outlined here supports a transient and dynamic binding mode for kwakhurin.

3.3.3 TP53-Hypoxyside A complex

The RMSD analysis of complex revealed that the system required a longer time to reach equilibrium. A gradual increase in RMSD was noted during the initial phase of the simulation: from ~1 to ~4 Å for the backbone and from ~1 to ~5 Å for the complex. In comparison to other complexes, the RMSD was not stable until some 200 ns in time, which required a very long simulation period to 500 ns. The total values of RMSD, especially of the complex (~5 Å), were more than those of *IGF1R* and *KRAS* systems. In the C-alpha atoms, the RMSFD values were between approximately ~0.6 Å and ~0.8 Å, respectively, but more so between approximately ~0.8 Å and ~1.2 Å, respectively, for the complex. It is noteworthy that the C-alpha RMSFD curves were almost identical and complex RMSFD curves were almost identical. The Rg values were shifting ~16.0 to ~17.5 Å and then stabilized after 150 ns. There were however two distinct fluctuations at around 190 ns and 300 ns. After stabilization of the complex more than 300 ns, fluctuations in Rg lowered and it was an indication of structural stability of the globe. The analysis of hydrogen bond indicated that during the simulation, 5 hydrogen bonds were maximum between Hypoxyside A and TP53. It is noteworthy that the first ~100 ns were always characterized by the presence of 3 hydrogen bonds. Between ~100 and ~150 ns the number rose to 5 and this suggests a stronger interaction. At a point beyond ~150 ns the count of hydrogen bonds returned to 3. The SASA values have decreased initially till ~75 ns then slowly increased between 105 and 115 nm². The values of the simulation during stabilization after stabilization varied between approximately 103 and 119 nm² with the curves of the complex and the protein almost overlapping. The TP53-Hypoxyside A complex monomethyl modification presents a per-residue energy

map pointing out the various energetic landscapes of the binding interface. Residues *TYR205* (−0.45 kcal/mol), *CYS187* (−0.41 kcal/mol), and *LEU137* (−0.39 kcal/mol) emerged as key stabilizing contributors. The involvement of *CYS238*, albeit with a slightly positive contribution (+0.44 kcal/mol), suggests a context-dependent role- possibly influenced by conformational constraints, solvent exposure, or proximity to flexible loop regions (Fig. 12, Movie 3).

3.4 Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) analysis

The MMPBSA analysis was performed using 1000 frames for all three systems: the *KRAS*–4-p-coumaroylquinic acid complex, the unbound *KRAS* receptor, and the free ligand.

3.4.1 KRAS 4-p-coumaroylquinic acid complex

For the *KRAS*- ligand complex, the molecular mechanics energy term values were as follows: bond energy was 571.92 ± 21.03 kcal/mol, angle energy 1313.20 ± 13.18 kcal/mol, dihedral energy 1730.00 ± 15.26 kcal/mol, improper torsion (IMP) 94.32 ± 10.06 kcal/mol, correction map (CMAP) −101.38 ± 7.33 kcal/mol, Urey-Bradley (UB) energy 190.92 ± 5.45 kcal/mol. Non bonded interaction term values were as follows- van der Waals energy (VDW AALS) −1148.77 ± 26.06 kcal/mol, electrostatic energy (EEL) −10,706.27 ± 87.07 kcal/mol, 1–4 van der Waals energy 487.36 ± 53.33 kcal/mol, and 1–4 electrostatic energy 6320.65 ± 53.33 kcal/mol. The solvation term values were as follows- the polar solvation energy (EPB) was −2730.92 ± 84.33 kcal/mol, nonpolar solvation energy (ENPOLAR) 1387.34 ± 7.84 kcal/mol, dispersion energy (EDISPER) −805.58 ± 13.41 kcal/mol. The gas-phase energy (GGAS) and solvation energy (GSOLV) were −1267.68 ± 110.57 kcal/mol and −2149.15 ± 85.75 kcal/mol, respectively. The total binding free energy was −3416.84 ± 139.92 kcal/mol.

For the *KRAS* receptor, the corresponding values were: bond energy 561.29 ± 21.17 kcal/mol, angle 1295.42 ± 13.37 kcal/mol, dihedral 1698.62 ± 13.44 kcal/mol, UB 167.85 ± 5.61 kcal/mol, IMP 93.89 ± 10.08 kcal/mol, CMAP −101.38 ± 7.33 kcal/mol, VDWAALS −1121.00 ± 23.47 kcal/mol, EEL −10,562.80 ± 58.19 kcal/mol, 1–4 VDW 469.79 ± 10.00 kcal/mol, 1–4 EEL 6170.63 ± 52.20 kcal/mol, EPB −2730.92 ± 82.77 kcal/mol, ENPOLAR 1370.98 ± 7.01 kcal/mol, EDISPER −804.41 ± 11.86 kcal/mol, GGAS −1327.68 ± 107.83 kcal/mol,

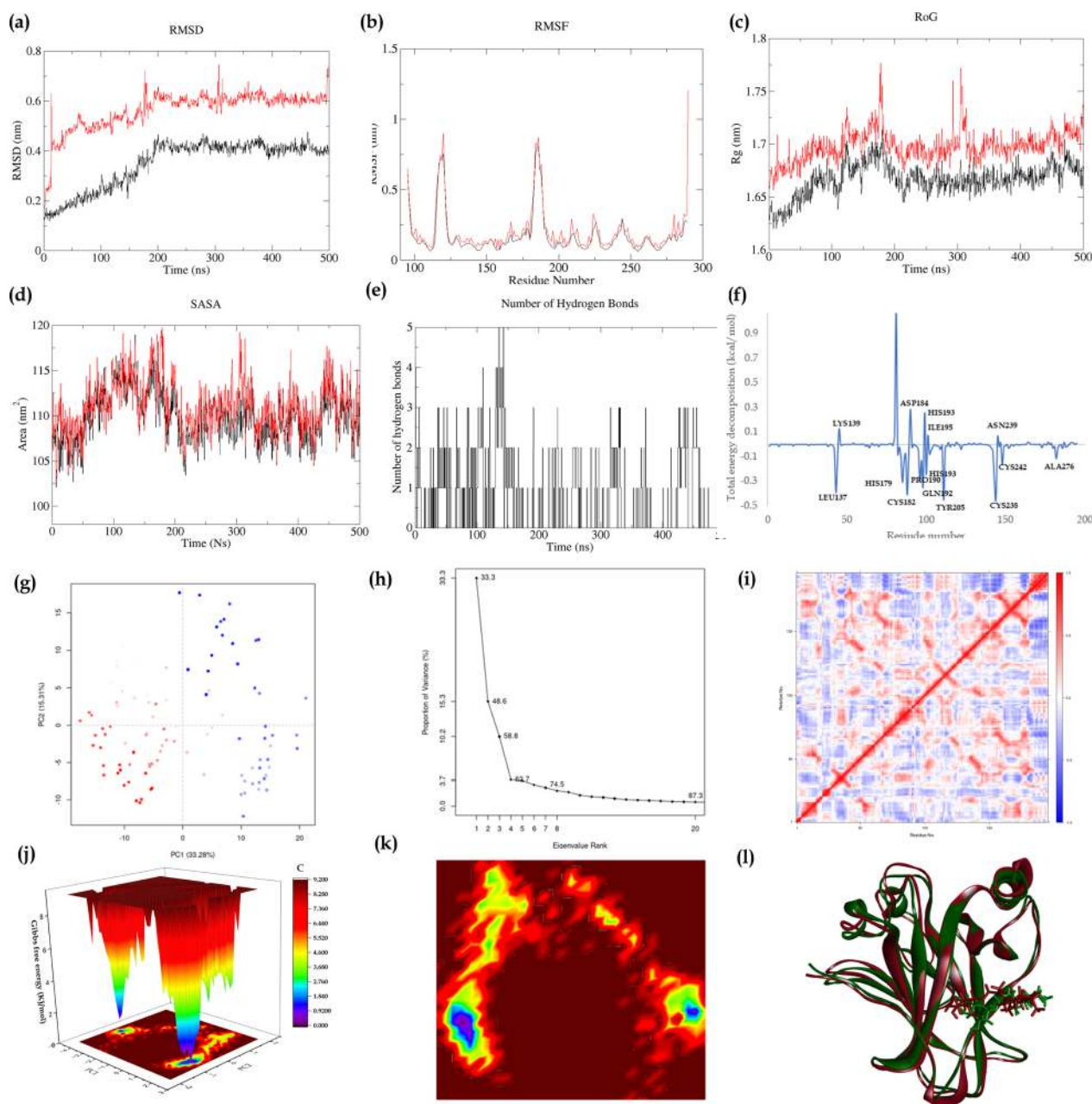


Fig. 12 Parameters describing the stability of complex *TP53*- Hypoxoside A complex **a** RMSD of backbone (black) and complex (red) **b** RMSF for c-alpha atoms (black) and complex (red) **c** RoG of backbone (black) and complex (red) **d** SASA of protein (black) and complex (red) **e** Number of hydrogen bonds formed **f** Total energy decomposition per residue **g**, **h** Principal component analysis (PCA) of motion projection plot for amino acid C- α atoms in phase space along the PC1 and PC2 and eigenvector rank plot using first 20 eigenvectors value. The continuous color spectrum of dots from blue to white to red signifies the MD simulation time scale (0–200 ns). The initial, intermediary, and finishing timescales are represented by blue, white, and red, respectively **i** Dynamic cross- correlation matrix (DCCM) plots for amino acid residues present in the three complex dynamic systems **j** 3D plot free energy landscape projection using PC1 and PC2 **k** 2D plot of free energy landscape projection using PC1 and PC2 **l** 3D interaction of complex with ligand depicted as green (start of simulation) and blue (end of simulation) and surface on the 3D interaction with respect to hydrogen bond donors and acceptors. Where, different colors on protein represent different residues

GSOLV – 2173.33 ± 83.91 kcal/mol, and the total energy – 3501.01 ± 136.63 kcal/mol.

For the ligand (4-p-coumaroylquinic acid), the bond energy was 10.63 ± 1.84 kcal/mol, angle energy 17.82 ± 2.21 kcal/mol, dihedral 31.72 ± 2.64 kcal/mol, UB 3.07 ± 0.65 kcal/mol, IMP 0.43 ± 0.39 kcal/

Table 2 Energy decomposition values from MMPBSA analysis for complex, receptor and ligand

Component	Complex (kcal/mol)	Receptor (kcal/mol)	Ligand (kcal/mol)
<i>KRAS-4-p-coumaroylquinic acid</i>			
GGAS	-1267.68 ± 110.57	-1327.68 ± 107.83	112.45 ± 10.60
GSOLV	-2149.15 ± 85.75	-2173.33 ± 83.91	-32.10 ± 2.05
Total free energy	-3416.84 ± 139.92	-3501.01 ± 136.63	80.35 ± 10.80
<i>IGF1R- Kwakhurin complex</i>			
GGAS	-2217.58 ± 153.27	-2239.75 ± 152.15	80.35 ± 6.68
GSOLV	-3837.00 ± 113.49	-3875.24 ± 110.85	-25.83 ± 2
Total free energy	-6054.58 ± 190.72	-6114.99 ± 188.25	54.52 ± 6.98
<i>TP53- Hypoxoside A complex</i>			
GGAS	-2725.85 ± 158.61	-2904.6 ± 167.1	211.02 ± 13.47
GSOLV	-1772.1 ± 111.37	-1781.78 ± 121.64	-25.23 ± 3.7
Total free energy	-4497.95 ± 193.81	-4686.38 ± 206.68	185.8 ± 13.97

GGAS: Gas Phase Energy; GSOLV: Solvation Free Energy

mol, CMAP 0.00, VDWAALS -2.47 ± 1.18 kcal/mol, EEL -116.33 ± 7.81 kcal/mol, 1-4 VDW 17.57 ± 1.49 kcal/mol, 1-4 EEL 150.01 ± 5.65 kcal/mol, EPB -33.48 ± 2.03 kcal/mol, ENPOLAR 35.86 ± 0.23 kcal/mol, EDISPER -34.49 ± 0.13 kcal/mol, GGAS 112.45 ± 10.60 kcal/mol, GSOLV -32.10 ± 2.05 kcal/mol, resulting in a total energy of 80.35 ± 10.80 kcal/mol.

Table 2 shows energy decomposition values from MMPBSA analysis for *KRAS*-4-p-coumaroylquinic acid complex, *KRAS* receptor, and free ligand.

Using the standard thermodynamic cycle:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{receptor}} + G_{\text{ligand}})$$

$$\Delta G_{\text{bind}} = -3416.84 - (-3501.01 + 80.35) = 3.82 \text{ kcal/mol}$$

The MMPBSA analysis indicates a moderate binding affinity between 4-p-coumaroylquinic acid and *KRAS*, with a calculated binding free energy of 3.82 kcal/mol. Though not extremely high in magnitude, this energy reflects a favorable and spontaneous interaction in the simulated environment.

3.4.2 IGF1R—Kwakhurin complex

For the *IGF1R*-Kwakhurin complex, the energy terms were as follows: bond energy was 1011.98 ± 19.16 kcal/mol, angle energy 2397.47 ± 27.36 kcal/mol, dihedral energy 1650.94 ± 23.42 kcal/mol, IMP 161.71 ± 7.15 kcal/mol, CMAP correction -432.45 ± 13.01 kcal/mol, UB energy 301.27 ± 8.77 kcal/mol, VDWAALS -2130.37 ± 20.11 kcal/mol, EEL -18,156.82 ± 130.05 kcal/mol, 1-4 van der Waals energy 906.65 ± 14.05 kcal/mol, and 1-4 electrostatic energy -18,156.82 ± 130.05 kcal/mol. The EPB was -4960.75 ± 111.70 kcal/mol, nonpolar solvation energy ENPOLAR 2509.76 ± 11.98 kcal/

mol, EDISPER -1386.02 ± 16.13 kcal/mol. The GGAS and GSOLV were -2217.58 ± 153.27 kcal/mol and -3837 ± 113.49 kcal/mol, respectively. The total binding free energy -6054.58 ± 190.72 kcal/mol.

For the *IGF1R* protein, the energy terms were as follows: bond energy was 995 ± 17.89 kcal/mol, angle energy 2374.19 ± 26.52 kcal/mol, dihedral energy 1630.66 ± 22.54 kcal/mol, IMP 161.43 ± 7.13 kcal/mol, CMAP correction -432.45 ± 13.01 kcal/mol, UB energy 301.27 ± 8.77 kcal/mol, VDWAALS -2089.4 ± 20.96 kcal/mol, EEL -18,140.44 ± 129.3 kcal/mol, 1-4 van der Waals energy 865.14 ± 14.65 kcal/mol, and 1-4 electrostatic energy 12,094.85 ± 62.83 kcal/mol. The EPB was -4973.74 ± 109.06 kcal/mol, ENPOLAR 2497.25 ± 12.63 kcal/mol, EDISPER -1398.75 ± 15.35 kcal/mol. The GGAS and GSOLV were -2239.75 ± 152.15 kcal/mol and -3875.24 ± 110.85 kcal/mol, respectively. The total binding free energy was found to be -6114.99 ± 188.25 kcal/mol.

For the Kwakhurin Ligand, the energy terms were as follows: bond energy was 16.98 ± 2.43 kcal/mol, angle energy 23.28 ± 2.35 kcal/mol, dihedral energy 20.29 ± 3.15 kcal/mol, IMP 0.28 ± 0.05 kcal/mol, VDWAALS -3.41 ± 1.97 kcal/mol, EEL 4.23 ± 3.65 kcal/mol, 1-4 van der Waals energy 41.51 ± 2.14 kcal/mol, and 1-4 electrostatic energy -22.81 ± 1.24 kcal/mol. The EPB was -28.4 ± 1.83 kcal/mol, ENPOLAR 39.99 ± 0.58 kcal/mol, EDISPER -37.43 ± 0.58 kcal/mol. The gas-phase GGAS and GSOLV were 80.35 ± 6.68 kcal/mol and -25.83 ± 2 kcal/mol, respectively. The total binding free energy was 54.52 ± 6.98 kcal/mol.

Table 2 shows energy decomposition values from MMPBSA analysis for *IGF1R*-Kwakhurin complex, *IGF1R* receptor, and free ligand.

The binding free energy (ΔG_{bind}) was +53.43 kcal/mol, suggesting an unfavorable or weak binding interaction,

3.4.3 TP53-Hypoxoside A complex

For the TP53 complex, the energy terms were as follows: bond energy was 622.49 ± 13.18 kcal/mol, angle energy 1526.51 ± 40.75 kcal/mol, dihedral energy 1134.37 ± 17.99 kcal/mol, IMP 95.13 ± 7.18 kcal/mol, CMAP correction -101.38 ± 7.33 kcal/mol, UB energy 184.84 ± 3.42 kcal/mol, VDWAALS -1258.41 ± 15.77 kcal/mol, EEL $-10,160.7 \pm 141.76$ kcal/mol, 1–4 van der Waals energy 474.08 ± 10.27 kcal/mol, and 1–4 electrostatic energy 4943.1 ± 46.81 kcal/mol. The EPB was -2427.14 ± 110.31 kcal/mol, ENPOLAR 1596.56 ± 8.16 kcal/mol, dispersion energy EDISPER -941.52 ± 13 kcal/mol. The gas-phase energy GGAS and solvation energy GSOLV were -2725.85 ± 158.61 kcal/mol and -1772.1 ± 111.37 kcal/mol, respectively. The total binding free energy was calculated to be -4497.95 ± 193.81 kcal/mol.

For the TP53 protein, the energy terms were as follows: bond energy was 610.35 ± 13.17 kcal/mol, angle energy 1480.27 ± 38.57 kcal/mol, dihedral energy 1115.53 ± 18.93 kcal/mol, IMP 95.11 ± 7.19 kcal/mol, CMAP correction -287.28 ± 17.2 kcal/mol, UB energy 184.84 ± 3.42 kcal/mol, VDWAALS -1237.84 ± 16.25 kcal/mol, EEL $-10,043.7 \pm 150.68$ kcal/mol, 1–4 van der Waals energy 462.02 ± 10.08 kcal/mol, and 1–4 electrostatic energy 4716.13 ± 49.71 kcal/mol. The EPB was -2421.57 ± 120.78 kcal/mol, ENPOLAR 1568.55 ± 7.76 kcal/mol, EDISPER -928.76 ± 12.18 kcal/mol. The GGAS and solvation energy GSOLV were -2904.6 ± 167.1 kcal/mol and -1781.78 ± 121.64 kcal/mol, respectively. The total binding free energy was calculated to be -4686.38 ± 206.68 kcal/mol.

For hypoxoside ligand, the energy terms were as follows: bond energy was 12.13 ± 4.45 kcal/mol, angle energy 46.24 ± 7.29 kcal/mol, dihedral energy 18.83 ± 3.71 kcal/mol, IMP 0.02 ± 0.02 kcal/mol, VDWAALS 0.15 ± 1.72 kcal/mol, EEL -105.39 ± 7.38 kcal/mol, 1–4 van der Waals energy 12.06 ± 1.21 kcal/mol, and 1–4 electrostatic energy 226.97 ± 5.98 kcal/mol. The polar solvation energy EPB was -27.54 ± 3.54 kcal/mol, ENPOLAR 43.65 ± 0.6 kcal/mol, EDISPER -41.33 ± 0.87 kcal/mol. The GGAS and GSOLV were 211.02 ± 13.47 kcal/mol and -25.23 ± 3.7 kcal/mol, respectively. The total binding free energy was calculated to be 185.8 ± 13.97 kcal/mol.

Table 2 represents energy decomposition values from MMPBSA analysis for TP53–Hypoxoside A complex, TP53 receptor, and free ligand.

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{receptor}} + G_{\text{ligand}})$$

$$\Delta G_{\text{bind}} = -4497.95 - (-4686.38 + 185.8) = 2.63 \text{ kcal/mol}$$

This positive binding free energy of 2.63 kcal/mol suggests that the interaction between Hypoxoside A and TP53 may not be thermodynamically favorable under the simulated conditions.

3.5 Principal component analysis (PCA)

PCA was performed to identify the dominant conformational motions within the molecular dynamic's trajectories of the KRAS- 4-p-coumaroylquinic acid, IGF1R- Kwakhurin, and TP53–Hypoxoside A complexes. The eigenvalues derived from the covariance matrix of atomic positional fluctuations were used to evaluate the proportion of motion captured by each principal component (PC). For the KRAS- 4-p-coumaroylquinic acid complex, the first three principal components- PC1, PC2, and PC3- accounted for 14.88%, 11.08%, and 7.85% of the total motion, respectively. The percentage of motion in the IGF1R- Kwakhurin complex was much higher with PC1 (35.84%) and then with, PC2 (11.6%) and PC3 (8.12%). PC1, PC2, and PC3 provided 33.28, 15.21 and 10.18 percent of the total motion in the case of the TP53–Hypoxoside A complex.

4 Discussion

The study evaluated the possibility of *S. trilobatum* bioactives in anti-breast cancer dynamics by employing a systems biology framework, such as gene set enrichment, gene ontology and simulations through molecular dynamics. *S. trilobatum* was researched to have 8 synonyms implying that it has had varied taxonomic bases and distribution spread. In all reported synonyms 425 bioactives were discovered, which is a rich source of drugs of discovery.

The screening and ADMET profiling drug-likeness of these bioactives was very valuable in giving a clue to their therapeutic value. Out of the 425 bioactives, 205 had a positive score of drug-like with josamycin scoring highest of 1.46- with an indication of good drug-like behaviours. Besides, 65 compounds exhibited zero violations of Lipinski Rule of Five, which involves positive characteristics of oral bioavailability. It was found that 168 compounds show anti-neoplastic properties with a specific effect on breast cancer, by using biological spectrum analysis, and this fact indicates

that the given bioactive pool is oncologically relevant. Considering both, 16 bioactives based on drug-likeness and therapeutic efficacy were shortlisted which was a necessary process in selecting candidates to further research.

These 16 compounds were profiled using ADMET where a range of pharmacokinetic properties was identified. The 22a,23a-epoxy-solanida-1,4,9-trien-3-one and 5-hydroxy-7-methoxy-3-(3-methoxyphenyl)-8-methyl-4H-chromen-4-one showed a great intestinal absorption, implying that they have high chances of being active systemically. Hypoxyside A exhibited a high level of interaction with P-glycoprotein and CYP3A4 that are important in drug metabolism. This means that they have a potency as a modulator in multidrug resistance mechanisms. In the same manner, the cytochalasin Z4 and 5, 6-epoxy-phomol have an intense interaction with enzymes that can be case strategically to affect the degradation of drugs and enhance therapeutic targeting. 4- p -coumaroylquinic acid and baragenin were interesting in that they have a high CYP450 enzyme inhibitory effect. Although it indicates that toxicity and interaction analysis will have to be approached carefully, these properties can also be altered and studied to be used therapeutically. For instance, CYP450 inhibitors are typically used as adjuvants to extend the half-life of concomitant medications, which might decrease drug dosage frequency and enhance efficacy.

The conducted work on target identification and analysis enrichment allowed understanding the molecular landscape that was regulated by the phytoconstituents of *S. trilobatum* in depth. The therapeutic potential of the plant is indicated by the 2,141 identified breast cancer-related targets and the following prediction of 531 protein targets regulated by 16 bioactive compounds. It is important to note that these targets dysregulated various major signaling pathways that are becoming widely deregulated in breast cancer such as PI3K-Akt, MAPK, Estrogen, Wnt, and mTOR pathways, which are known to mediate the process of tumor proliferation, angiogenesis, metastasis, and resistance mechanisms [35–38]. The targets were also linked with signaling pathways including the endocrine resistance, HIF-1 and the apoptosis pathways implying that the bioactives have potential beyond the tumor progress suppression as well as sensitizing cells to the currently available therapeutic agents [39]. Particularly, the PI3K-Akt signaling pathway emerged as the most dominantly enriched, indicating its centrality in the network. Its role is well established in tumor growth, survival, and therapeutic resistance [40].

Notably, many of these enriched pathways such as Estrogen signaling, endocrine resistance and PI3K-Akt, are closely related to steroid hormone receptor biology.

Their leading role in the enrichment analysis may indicate that *S. trilobatum* bioactives may act in part through networks that are associated with estrogen and progesterone receptor activation, to thus have an effect on steroid receptor-linked oncogenic circuitry.

Further, network pharmacology analysis identified 10 key hub genes- *AKT1*, *TP53*, *TNF*, *STAT3*, *MYC*, *IL6*, *HIF1A*, *EGFR*, *CTNNB1*, and *CASP3*- all of which have extensive evidence supporting their roles in the pathogenesis of breast cancer. For instance, *AKT1* is pivotal for cell survival and metabolism [41], *TP53* governs genomic stability and apoptosis [42], while *EGFR* and *CTNNB1* have an important role to play in proliferative and cell differentiation [43, 44]. Notably, rheochrysin appeared as a central modulator with strong topological influence, while 7,2'-dihydroxy-4'-methoxyisoflavanol emerged as a peripheral, possibly playing a supplementary role. 3a,16a-Dihydroxyandrostene, with the highest betweenness centrality, may act as a crucial linker across subnetworks [45]. All of these findings suggest the regulatory multi-target nature of *S. trilobatum* constituents in oncogenic pathways.

The functional relevance of the target genes was also confirmed by GO enrichment analysis. The receptor complex was enriched more than any other CC category, especially around the regulation of a number of different transmembrane receptor tyrosine kinases, which underlines the potential interception of upstream signalling events. Protein binding was the most largely enriched in MF category indicating that the majority of the modulated target functions determined as scaffolds or interactors in intricate signaling cascades. The response to chemical was heavily enriched in BP domain, which implies a global modulation of chemoresistance stress responses genes- drug metabolism genes- an important consideration in developing chemoresistant resolutions. The combined analysis of KEGG and GO databases showed that 90.8 percent of genes are in common confirming the high degree of functional consistency between the biological roles and the pathway associations of the discovered targets. It means that the bioactives that are obtained in *S. trilobatum* do not only regulate the diverse pathways but have a range of other regulated biological networks that provide a holistic mode of action against breast cancer. Figure 13 demonstrates the molecular mechanism of action of the bioactives in *S. trilobatum* that is predicted to be used in treating breast cancer.

The bindings noted in the molecular docking experiments can provide important information as to whether bioactives have the potential of regulating important cancer-related targets. Of particular interest

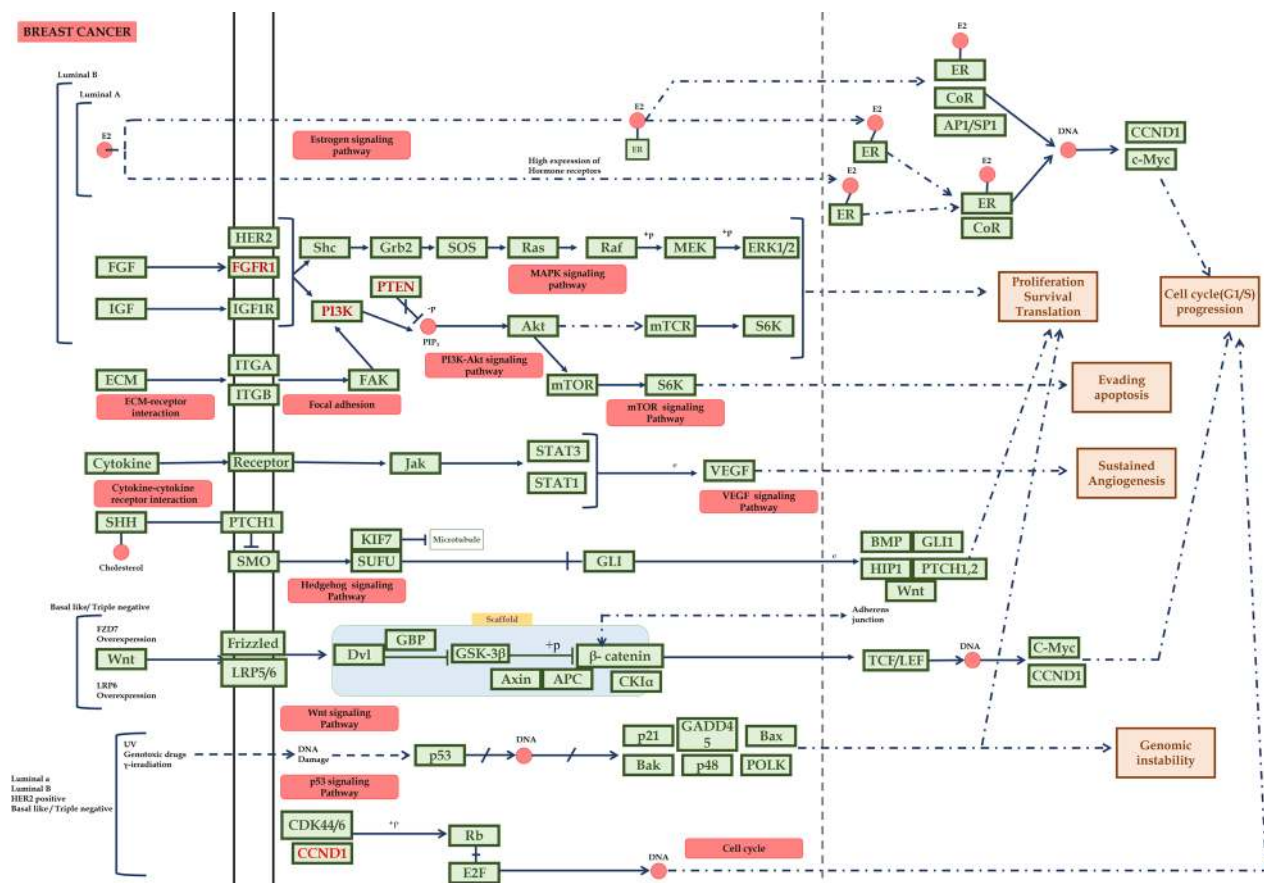


Fig. 13 Proposed possible molecular mechanism of screened bioactives in breast cancer. Indicates genes identified to be modulated by the screened bioactives. The figure highlights regulation of estrogen receptor-mediated transcription, growth factor signaling (HER2/EGFR, IGF1R), and downstream MAPK and PI3K-AKT-mTOR pathways that control proliferation, survival, and metabolism. Modulation of TP53-associated DNA damage and cell-cycle checkpoint pathways, along with Wnt/β-catenin, Hedgehog, and JAK/STAT signalling, is shown to influence apoptosis, angiogenesis, genomic stability, and cell-cycle progression

is the interaction of bioactives with RAS proteins (HRAS and KRAS) which in most cancers are mutated resulting in unregulated cell proliferation [46]. Inhibition of KRAS has similarly been an accepted problem since it has a smooth surface and no classical binding sites [47]; Therefore, by revealing natural stable-receptors to bind to KRAS, such as 4-p-coumaroylquinic acid and hypoxiside, there exists opportunity of anti-cancer intervention. On the same note, the PI3K pathway is one of the pathways that emerged as a pivotal node that the interaction with PIK3CB, which is a subunit of the PI3K catalytic is capable of inducing also reveals the possibilities of bioactives to interfere with key signalling cascades that determine tumor survival, angiogenesis, and apoptotic resistance [41]. Moreover, the thing is that bioactives are bound to nuclear targets (such as NCOA3 and PGR), which may be involved in the regulation of hormone-responsive breast cancer pathways. NCOA3 is a transcriptional coactivator, which increases the

estrogen and progesterone receptor signalling and it is commonly over expressed in aggressive cancers [48]. Cytochalasin and rheochrysin compounds can interfere with the transcriptional activation of this group of genes by binding to NCOA3 and PGR. Also, there are IGF1R and TP53 which are two diametrically opposite nodes of cancer where IGF1R supports growth and survival, and TP53 is a tumor suppressor that controls cell cycle arrest and apoptosis [49–51]. These targets and ligands like kwakhurin and hypoxiside A indicate a possible growth and tumor-suppressive effect of *S. trilobatum* constituents. All in all, this multi-target interaction profile highlights their ability to be used in the therapy of breast cancer to regulate the complicated biology.

A combination of these interactions with PGR, NCOA3 and upstream regulators of the estrogen-linked pathways is powerful evidence that *S. trilobatum* bioactives can be used to regulate steroid receptor-coactivator

interface-interactions, and in downstream proliferative signalling pathways.

Considering several parameters of molecular simulation, the KRAS-4- p-coumaroylquinic acid complex turns out to be the most dynamically consistent and structurally stable system among the three analyzed complexes. The high convergence in the RMSD profile at an early stage, a large radius of gyration which reflects tightness and low values of the RMSF which shows low atomic fluctuations, underline its suitability in creating a high-affinity interaction and a specific interaction with minimal conformational disturbance. Conversely, it was observed that the TP53- Hypoxoside A complex was initially structurally unstable though it was able to attain more stable hydrogen bonding throughout the simulation. Such behaviour can suggest a weak but late binding interaction, which could prove useful in situations where long-term modulation is needed, and not instant inhibition. IGF1R-Kwakhurin complex exhibited intermediate behaviour with a certain amount of structural variability and partial recovery which indicated moderate dynamic stability. Nevertheless, the MMPBSA result gave a positive ΔG of +53.43 kcal/mol, of IGF1R-Kwakhurin complex which allows weak and undesirable binding. This is to imply that although Kwakhurin might possess initial affinity, yet structural enhancement or other binding might be necessary, to enhance its interaction with IGF1R.

The PCA output showed that the three protein–ligand complexes had significant variation in terms of most dominant motion profiles. The relative distribution of motion by the KRAS- 4-p-coumaroylquinic acid complex over several key components indicated a more rigid and stable complex, and less comprehensive conformational changes during the simulation. This is associated with its initial convergence, lowest RMSD variation, and desirable binding energetics, which makes it the most dynamically consistent system. On the other hand, the two complexes (IGF1R- Kwakhurin and TP53- Hypoxoside A) were more motions with higher per cent of movement of 35.84 and 33.28 in PC1 respectively, and pointed towards large-scale, collective domain movements. This could indicate a more flexible behaviour but can also indicate a weaker binding specificity when binding as it forms a comparatively weaker binding specificity in MMPBSA with IGF1R- Kwakhurin. Overall, PCA proves that KRAS-4-p-coumaroylquinic acid is the most conformationally stable and the least essential motions when it comes to its complex with TP53 and IGF1R, and these two coexist with an even greater conformational change. These dynamic features confirm the findings of

RMSF and MMPBSA data that is a complete description of the interactions between the ligand and the receptor.

The integration of network pharmacology, molecular docking, and molecular dynamics (MD) simulations identified TP53, KRAS, and IGF1R as pivotal protein nodes through which *S. trilobatum* bioactives may exert antineoplastic effects in breast cancer. These targets are central regulators of tumour initiation, progression, and therapeutic resistance and have been extensively validated in experimental and clinical studies [52–54]. Among the evaluated protein–ligand complexes, the TP53-Hypoxoside A complex displayed exceptional structural stability, suggesting a strong and sustained interaction under dynamic conditions. This observation is particularly significant, as TP53 remains one of the most critical tumour suppressors in oncology and a long-standing therapeutic challenge [55, 56].

TP53 functions as a molecular “rheostat” that integrates cellular stress signals and orchestrates DNA repair, cell-cycle arrest, senescence, and apoptosis [55, 57]. In breast cancer, TP53 is the most frequently altered gene, with mutations present in approximately 30% of all cases, increasing to >80% in aggressive subtypes such as triple-negative breast cancer (TNBC) and high-grade tumours [52, 53]. These alterations are strongly associated with genomic instability, poor prognosis, and resistance to therapy [58]. Importantly, many TP53 alterations represent gain-of-function (GOF) mutations, which not only abrogate tumour-suppressive activity but actively promote epithelial-mesenchymal transition (EMT), metabolic reprogramming, invasion, and metastasis [59–61]. In this context, the high conformational stability of the TP53–Hypoxoside A complex observed in our MD simulations is biologically meaningful. The sustained binding free energy, stable radius of gyration, and consistent RMSD convergence suggest that Hypoxoside A may stabilize the TP53 protein scaffold. Such stabilisation raises the possibility that Hypoxoside A could act as a pharmacological chaperone, potentially restoring wild-type-like conformational behaviour in destabilised TP53 variants, a strategy that has gained increasing attention in TP53-targeted cancer therapy [62].

Although KRAS mutations are less frequent in breast cancer compared to pancreatic or colorectal malignancies, aberrant activation of the RAS-MAPK signalling pathway is observed in a large proportion of advanced and metastatic breast tumours [63, 64]. KRAS functions as a molecular switch that transmits signals from upstream receptor tyrosine kinases to downstream effectors such as RAF, MEK, and ERK, thereby driving proliferation and survival [65]. In breast cancer, KRAS-driven signalling frequently acts as a bypass mechanism underlying therapeutic resistance, particularly when

primary targets such as HER2 or the estrogen receptor are inhibited [66, 67]. Targeting KRAS-associated signalling may therefore disrupt this compensatory proliferative axis. The interaction between Hypoxoside A and KRAS identified in our docking analysis provides a mechanistic basis for the ethnomedicinal use of *S. trilobatum* in limiting tumour growth and progression, suggesting potential utility in sensitizing resistant tumours to conventional therapies.

The insulin-like growth factor 1 receptor (IGF1R) is a well-established driver of breast cancer survival and a key mediator of endocrine resistance, particularly in estrogen receptor-positive (ER+) tumours [68, 69]. IGF1R signalling exhibits extensive cross-talk with estrogen receptor pathways, enabling tumour cells to maintain growth and survival even under estrogen deprivation or anti-estrogen therapy [70]. In addition, IGF1R robustly activates the PI3K-AKT-mTOR pathway, promoting anti-apoptotic signalling, protein synthesis, and metabolic adaptation [71, 72]. The identification of IGF1R as a significant target in our network pharmacology and docking analyses suggests that *S. trilobatum* bioactives may exert a dual inhibitory effect by attenuating both proliferative MAPK signalling (via KRAS) and survival-driven AKT signalling (via IGF1R). Such multi-pathway modulation is particularly relevant in breast cancer, where pathway redundancy and cross-talk often undermine single-target therapeutic strategies [73].

Collectively, these findings support a multi-target, pathway-oriented mechanism of action for *S. trilobatum* bioactives, with TP53, KRAS, and IGF1R serving as central molecular hubs. The pronounced stability of the TP53-Hypoxoside A complex further highlights TP53 as a promising lead target. Nonetheless, given the computational nature of the present study, these results should be considered hypothesis-generating, and experimental validation is required to confirm target engagement and therapeutic relevance.

5 Conclusion

The study aimed to elucidate the molecular processes that would explain how the bioactives of *Solanum trilobatum* can have its therapeutic action against breast cancer. The study started with a massive phytochemical study that identified 425 bioactives with 16 being shortlisted on criteria of drug-like properties and potentially anti-cancer properties as predicted. Later analysis of ADMET screening and network pharmacology houses these compounds to have a diverse array of targets linked with important pathways in breast cancer. Particularly, the PI3K-Akt signalling pathway has become the most enriched, which indicates that the bioactives could inhibit cell survival, proliferation, and resistance mechanisms

on a molecular scale. These interactions were further confirmed, molecular docking and dynamics simulations and 4-p-coumaroylquinic acid evolved into a relatively fixed complex with KRAS- a notorious cancer target in history- with other compounds also demonstrating an affinity to regulatory proteins such as TP53, IGF1R, and NCOA3. The presence of NCOA3 and PGR in the list of modulated targets, alongside the enrichment of PI3K-Akt signalling, indicated that NCOA3 and PGR may be manipulated by *S. trilobatum* bioactives to regulate steroid receptor-related molecular pathways in breast cancer. These findings not only support the multi-target therapeutic promise of *S. trilobatum* but also position it as a potential source of lead candidates for drug development.

Overall, the integrative computational framework employed in this study suggests that *S. trilobatum* bioactives may modulate oncogenic genes associated with steroid hormone receptor-related signalling, thereby providing a mechanistic basis for its potential role as a complementary therapeutic agent. Importantly, these findings are derived from in silico predictions and should be interpreted as hypothesis-generating. Consequently, comprehensive experimental validation, including in vitro and in vivo studies as well as dose-response correlation analyses, will be essential to confirm the predicted interactions, establish pharmacological relevance, and advance these observations toward clinical translation.

Abbreviations

BC	Breast cancer
BP	Biological process
CC	Cellular component
GO	Gene ontology
ER	Estrogen receptor
FDR	False discovery rate
GC	Gene count
KEGG	Kyoto encyclopedia of genes and genomes
MD	Molecular dynamics
MF	Molecular function
MMPBSA	Molecular Mechanics Poisson-Boltzmann Surface Area
NBHA	Number of hydrogen bond acceptor
NHBD	Number of hydrogen bond donor
PDB	Protein Data Bank
Rg	Radius of gyration
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
RCSB	Research collaboratory for structural bioinformatics
SMILES	Simplified molecular input line entry system
XP	Extra precision

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43088-026-00756-9>.

Additional file1 (MP4 6838 KB)

Additional file2 (MP4 8475 KB)

Additional file3 (MP4 7388 KB)
 Additional file4 (XLSX 121 KB)
 Additional file5 (XLSX 30 KB)
 Additional file6 (XLSX 276 KB)
 Additional file7 (XLSX 82 KB)
 Additional file8 (XLSX 65 KB)
 Additional file9 (DOCX 14 kb)

Author contributions

PSR contributed to the conceptualization of the study and was involved in data curation, formal analysis, investigation, methodology, software handling, visualization, and drafting of the original manuscript, in addition to supervision. MBA contributed to data curation, methodology development, validation, software analysis, and formal data analysis. WLL contributed to data curation, methodology development, validation, software analysis, and formal data analysis. CSS contributed to the conceptualization of the study and provided overall supervision, project administration, resource support, and critical review and editing of the manuscript. AN contributed to methodology development, software support, formal analysis, and visualization. AD contributed to methodology development, software support, formal analysis, supervision, and visualization. VLV contributed to manuscript review and editing, validation, interpretation of results, and resource support. PPP contributed to conceptualization, methodology, supervision, formal analysis, manuscript review and editing, project administration, and provided final approval of the manuscript.

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Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Human and animal rights

Not applicable.

Competing interests

The authors declare no competing interests.

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