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Extrapolations of Periwinkle Secondary Metabolites as Multitarget Inhibitor of Breast Cancer Proteins

Aparna Prasad*

Abstract

Objective: *Breast cancer is considered one of the most common and dangerous forms of cancer. It is a significant public health issue on a global scale. The study estimates that 10.0 million people will die from cancer this year, and 19.3 million people will develop the disease overall (BC). In this computation approach, nowadays emphasizes new drug discovery, the target protein epidermal growth factor receptor (EGFR) (5UGB), estrogen receptor (ER) (2QGT), and PI3KA (4OYS) were chosen to perform molecular docking against the derivatives of periwinkle Catharanthus phytochemical and FDA-approved drugs. Its pharmacological characteristics and its therapeutic analysis were investigated.* ***Methods:*** *The study was based on a computational approach using different phytocompounds for evaluation of their inhibitory potential against the target proteins EGFR, ER, and PI3KA. The protein molecular docking was conducted systematically using IMPPAT, PubChem, PDB, Open Bable, BIOVIA Discovery Studio Visualizer, PDB sum generate, PyRx, and ADMETlab 2.0.* ***Result:*** *The docking result revealed that the ligands selected have the best binding affinity with all three target proteins.* ***Conclusion:*** *The ligands could potentially be used to treat breast cancer in future approaches for studying the urge ligands in vitro and in vivo analysis to create novel breast cancer inhibitors.*

Keywords: Breast cancer, *Catharanthus*, periwinkle, EGFR, ER, PI3KA, vindolininol, lochnericine, lochnerinine, molecular docking, ADMET

INTRODUCTION

Cancer nowadays is the most common cause of death which is nothing but the stage where our body cells grow uncontrollably and has the potential to harm other regions of the body by spreading to those areas. worldwide it is one of the major public health issues according to researchers the estimation of cancer deaths is 10.0 million and the total number of new cancer cases is 19.3 million where breast cancer (BC) is the leading reason for cancer detected in females which is greatly exceeded lung cancer in terms of prevalently with a predicted 2.299 million instances of cancer diagnosed [1, 2].

***Author for Correspondence**
Aparna Prasad
E-mail: aparnaprasad699@gmail.com

Student, Department of Biotechnology, Pillai College of Arts, Commerce & Science (Autonomous) (affiliated to Mumbai University), New Panvel, Mumbai, Maharashtra, India

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Breast cancer is a sort of developing breast tissue cell carcinoma it is mostly grown on the innermost layer of the milk ducts (milk passageways), lobules (which transport the milk or glandular tissue house the milk-producing glands) a total of 10.4% of cancer is comprised of breast cancer among women which plays a key role in making it the fifth most frequent incidence of cancer death and the second most prevalent non-skin cancer type [3].

The incidence of breast cancer is influenced by several risk variables, such as demographic, reproductive, use of hormones, alcohol intake, genetics, obesity, nulliparity, breast-related, and

lifestyle factors. The risk of breast tumors is greatly influenced by factors related to a family history of the disease, factors related to atypical hyperplasia, and factors related to genetic predisposition [4, 5]. Although there are many alternative treatments available for breast cancer, in this study we specifically target the protein epidermal growth factor receptor (EGFR), estrogen receptor (ER), and PI3KA which are considered to cause breast cancer due to their mutation signaling pathways.

A transmembrane protein with an extracellular ligand binding domain, single transmembrane, and cytoplasmic tyrosine kinase domain, EGFR is a receptor tyrosine kinase (RTK) that is a member of the ErbB family. When breast cancer is triple-negative, it is typically overexpressed (TNBC). Through the activation of numerous downstream signaling pathways, including Ras-Raf-MEK-ERK, PI3K-AKT-mTOR, and Src-STAT3, receptor activation increases cell proliferation, motility, and survival [6]. All these substances are EGFR agonists, including the transforming growth factor alpha (TGF), epidermal growth factor (EGF), heparin-binding growth factor (HB-EGF), amphiregulin (AREG), epiregulin (EPI), epigen (EPG), betacellulin (BTC), and neuregulin (NRG) 2 [7]. The EGFR is, therefore, anti-EGFR treatment, including monoclonal antibodies (mAbs) and small molecule tyrosine kinase inhibitors (TKIs). ER is linked to breast cancer progression. For the mammary gland to develop and differentiate normally, estrogen is necessary. Additionally, it promotes the development of about 50% of initial breast tumors [8]. The TPs of ER-negative breast malignancies appear to be unique from those of ER-positive tumors, and their signaling is complicated, involving extranuclear activities and coregulatory proteins [9]. phosphatidylinositol 3-kinase (PI3K), also known as protein kinase B (PKB/AKT), mammalian target of the rapamycin (mTOR) signaling pathway. This protein inhibits cell proliferation, increases apoptosis, and decreases PI3K pathway indicators including pAkt and pPRAS40 for human PIK3CA mutant breast tumor cells [10]. In this study, the two FDA-approved drugs are used to compare their result with the phytocompounds of periwinkle (*Catharanthus*) standard drugs ribociclib and abemaciclib is mostly utilized in therapy to treat breast cancer patients to inhibit the growth of the cancerous tumor.

Catharanthus, popularly known as periwinkle, is an uncommon herb. It belongs to the Apocynaceae, it is commonly grown and native to Sri Lanka and India [11, 12]. Several of its common names are Vinca rosea, Madagascar, sparkling eyes, graveyard plant, Cape Periwinkle, old maid, graveyard, pink periwinkle, and Rose periwinkle myrtle [13]. These plants play a very important key role in synthesizing medicine as these a drugs from traditional systems the phytochemicals from the whole plant have the presence of alkaloids, phenol, flavonoids, steroids, protein, pain relieving properties, and most importantly anticancer properties.

The study's main objective is to assess the anticancer and inhibitory effects of the 21 selected phytocompounds from the periwinkle plant on the three target proteins of breast cancer EGFR, ER, and PI3KA. Also, to study the pharmacological as well as therapeutic efficacy, the results obtained for these studies will be compared to those commonly used drugs, ribociclib and abemaciclib which are approved by the FDA.

METHODS

Retrieval of Ligands

A total of 21 phytocompounds of periwinkle were derived from Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 (IMPPAT 2.0) (*https://cb.imsc.res.in/imppat/*). For further analysis. the canonical SMILES, PubChem CID, and two-dimensional (2D) models of these compounds along with two standard drugs were retrieved in SDF format using the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*) further all the structures in SDF files were then converted to PDB file format using a software called Open Babel.

Retrieval of Protein and Purification

The three-dimensional (3D) structure of proteins like EGFR (pdb Id:5UGB), ER (pdb Id:2qgt), and phosphatidylinositol 3-kinase (PI3K) (pdb Id: 4oys) was resolved using X-ray diffraction method with a resolution factor of 2.53Å, 2.15Å and 2.90Å respectively. The structures were downloaded using Research Collaboratory for Structural Bioinformatics PDB (RCSB PDB) (*https://www.rcsb.org/*) in pdb format.

The missing residues were fixed using BIOVIA where the protein is purified by deleting the water molecules HETATM and adding polar hydrogen to the retained chain A where the rest of the chains are deleted. This purified protein is then saved in a pdb file format which was used to obtain the two-dimensional structure and the Ramachandran plot using PDBsum generate (*https://bio.tools/pdbsum_generate*) and Hydrophobicity plot from BIOVIA Discovery Studio software.

Molecular Docking

Once the protein and ligand were retrieved molecular docking was executed using PyRx, it is mainly a virtual molecular screening application used to dock small molecular libraries to find lead compounds and their function. AutoDock Vina was used to perform molecular docking. It is a fully accessible virtual screening tool that can evaluate libraries of phytocompounds against a particular therapeutic target and is primarily used for computer-aided drug design (CADD) techniques. Here the selected 21 phytocompounds and two standard drugs were loaded as ligands and the three target proteins as macromolecules which are 5UGB, 2qgt, and 4oys. where the ligand loaded were energy minimized and was converted to .pdbqt form and the grid was generated for the targeted protein which is as follows the grid for the center is shown in Table 1 and the value obtained for grid dimension X: 25Å, Y: 25Å and Z: 25Å was similarly for all the three proteins.

All the ligands were docked with the target protein discretely and were evaluated based on binding affinity which is the strength of protein-ligand binding. that is the least binding means the best docking conformation (kcal/mol). The top three ligands with the least binding and zero root mean square deviation (RMSD) value were selected where the RMSD value is utilized to evaluate the docked conformation compared to other docked conformations and visualized the ligands were saved in pdb file format and the other docked conformation based on the binding affinity and the inhibitory activity of the ligands and standard drugs will be compared.

Visualization

The top three ligands having the least binding affinity for each protein were selected and the best model of each ligand was saved in pdb file format from PyRx. These were visualized using BIOVIA Discovery Studio software the three-dimensional (3D) structure and the non-bond interaction were observed, and the 3D model was extracted in png file format.

Physiochemical Studies (ADMET Analysis)

The pharmacokinetics were evaluated in ADMET (*https://admetmesh.scbdd.com/*) using Lipinski's rule of five (RO5). Pharmacodynamic properties were predicted by parameters such as physiochemical properties, absorption, distribution, metabolism, medical chemistry, toxicity, and excretion. The highest three docked ligands having the least binding affinity for each protein were evaluated ADMET analysis was performed using ADMETlab 2.0 (*https://admetmesh.scbdd.com/*).

Table 1. The details about the grid center.

PDB ID of protein	X	Y	Z
5UGB	7.9228	58.133	39.5791
2qgt	18.9175	10.7278	10.6316
4oys	35.5374	14.865	66.5805

RESULT

Selection of Phytocompounds

A total of 21 phytocompounds of periwinkle (*Catharanthus*) were chosen from IMPPAT out of which only eight phytocompounds were selected based on docking results and the two-dimensional (2D) chemical structure in Figure 1. To check the inhibitory activity of these compounds against the target proteins two standard drugs Abemaciclib and Ribociclib were retrieved which is shown in Figure 1.

Protein Retrieval and Purification

The three-dimensional (3D) crystal structure of the three target proteins was retrieved from PDB. After that, the proteins 5UGB, 2QGT, and 4OYS were purified in the BIOVIA Discovery software which is subjected to its structure analysis which is seen in Figures 2–4, respectively. In structural analysis, the Ramachandran plot, Secondary Structure, and hydropathy plot are analyzed.

The Ramachandran Plots

- *EGFR:* As shown in Figure 2(d) the 3D structure has 86.5% of residues in the region that are most preferred, 11.2% in the extra allowed region, 1.5% in the generously allowed region, and o.7% in the rejected region.
- *ER:* As shown in Figure 3(d) the 3D structure has 92.1% of residues in the region that are most preferred, 5.7% in the extra allowed region, 0.9% in the generously allowed region, and 1.3% in the rejected region.
- *PI3KA:* As shown in Figure 4(d) the 3D structure has 76.3% of residues in the region that are most preferred, 19.7% in the extra allowed region, 2.0% in the generously allowed region, and 2.0% in the rejected region.

Secondary Structure

- *EGFR:* As shown in Figure 2(c) its secondary structure predicts the presence of 2 sheets, 6 beta hairpins, 4 beta bulges, 9 strands, 31 helices, 51 helix-helix interfaces, 37 beta turns, and 4 gamma turns.
- *ER:* As shown in Figure 3(c), its secondary structure predicts the presence of 1 sheet, 1 beta-hairpin, 1 beta bulge, 2 strands, 11 helices, 20 helix-helix interfaces, and 15 beta turns.
- *PI3KA:* As shown in Figure 4(c) its secondary structure predicts the presence of 2 sheets, 4 beta hairpins, 4 beta bulges, 8 strands, 17 helices, 12 helix-helix interfaces, 30 beta turns, and 2 gamma turns.

Hydropathy Plot

An amino acid sequence hydrophobic and hydrophilic tendencies are shown on a hydropathy plot, a quantitative analysis. The hydropathy plot of all three target proteins EGFR, ER, and PI3KA was analyzed using BIOVIA Discovery Studio Visualizer which is shown in Figures 2(b), 3(b), and 4(b) respectively.

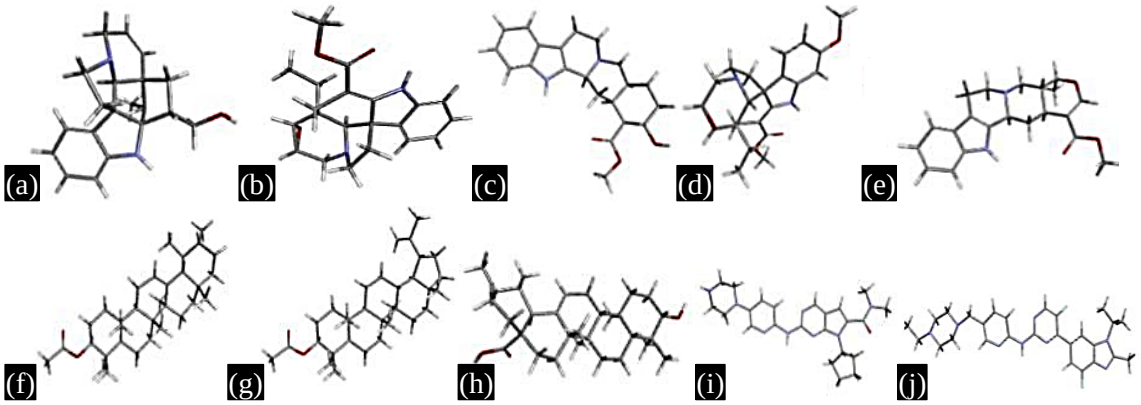


Figure 1. Chemical structure of top 8 phytocompounds and standard drugs; (a) vindolininol, (b) lochnericine, (c) lochnerinine, (d) ursolic acid, (e) alpha-Amyrenyl acetate, (f) rauwolscine, (g) lupeol acetate, (h) ajmalicine, (i) ribociclib and (j) abemaciclib.

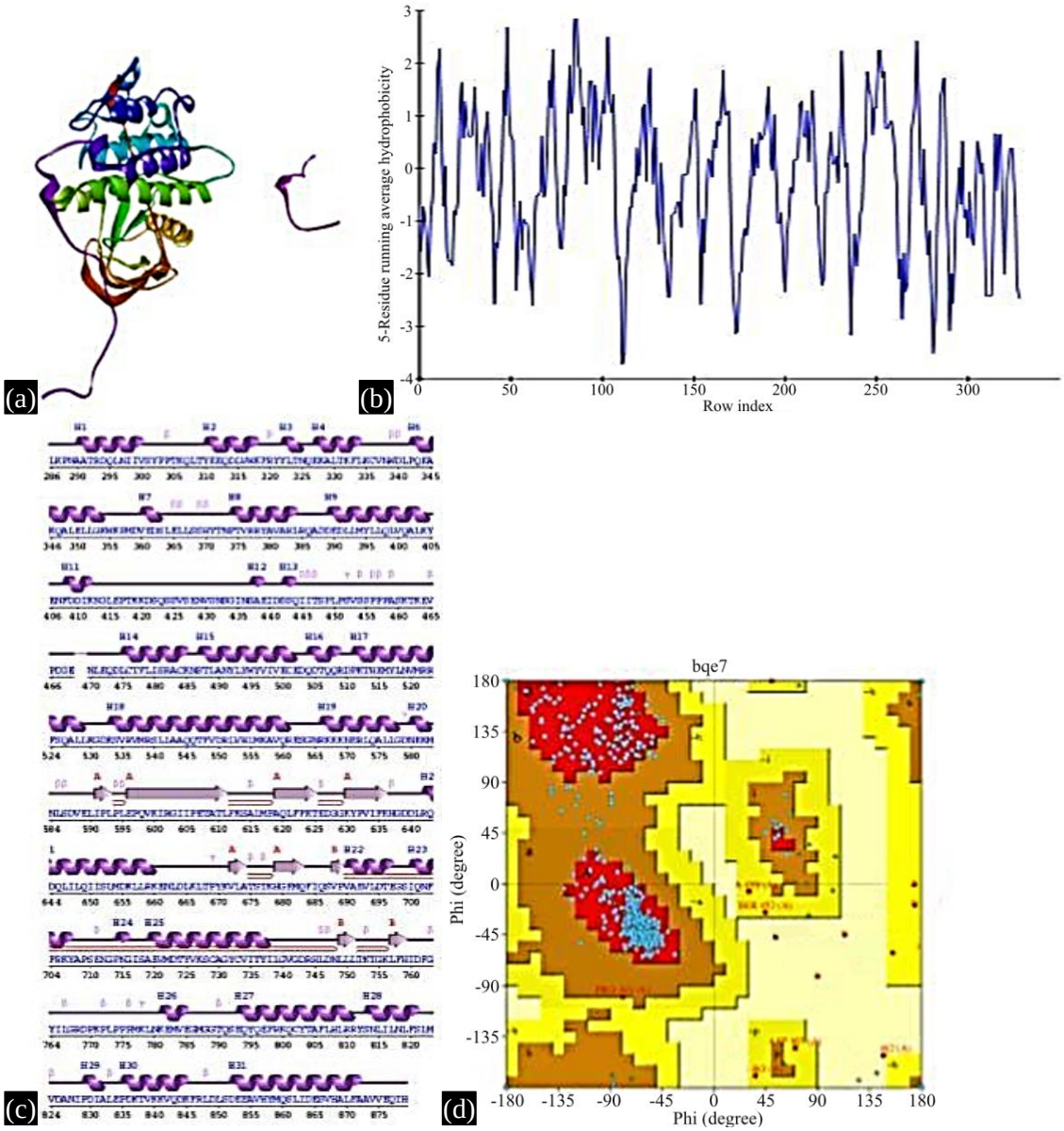
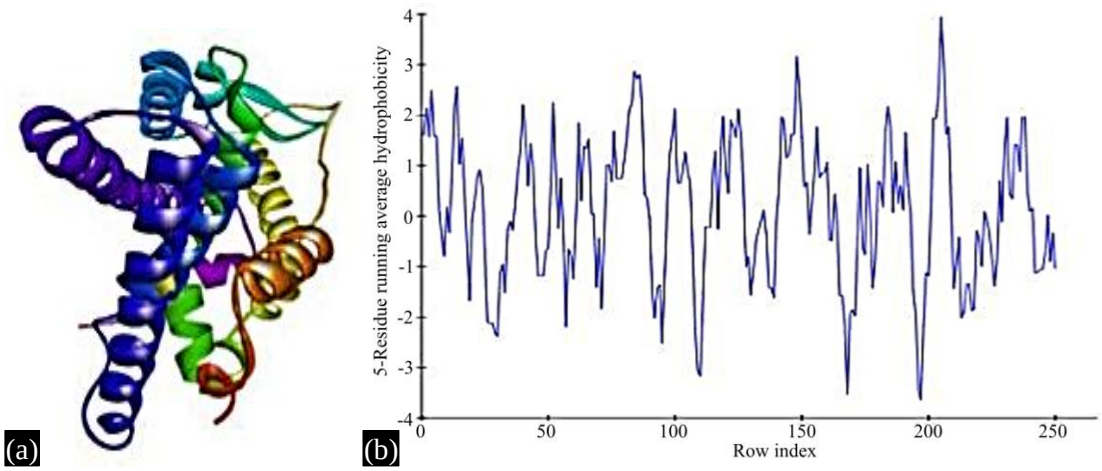


Figure 2. Structural analysis of EGFR (5UGB); (a) purified protein of 5UGB, (b) hydropathy plot, (c) predicted secondary structure, (d) Ramachandran plot.



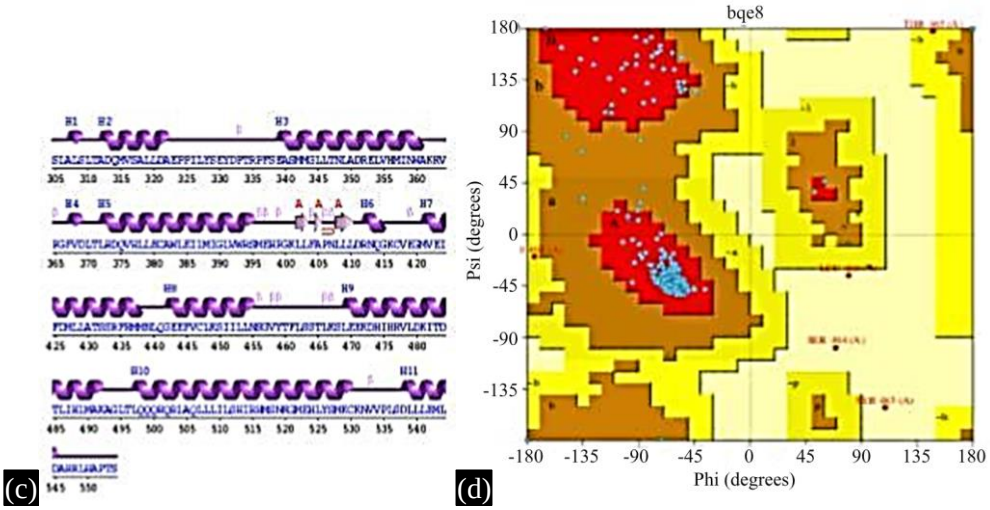


Figure 3. Structural analysis of ER (2QGT); (a) purified protein of 2QGT, (b) hydropathy plot, (c) predicted secondary structure, (d) Ramachandran plot.

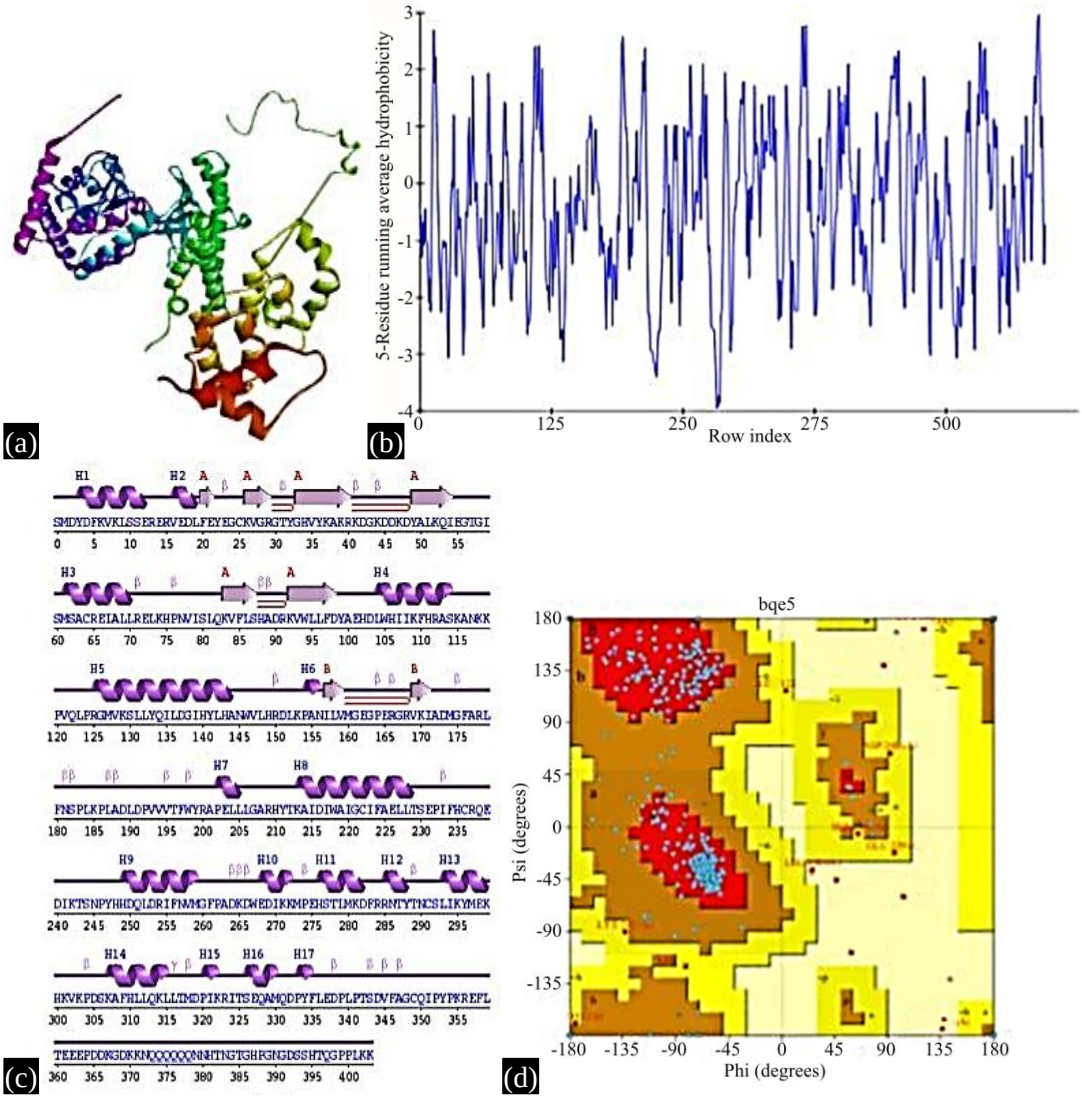


Figure 4. Structural analysis of PI3KA (4OYS); (a) purified protein of 4OYS, (b) hydropathy plot, (c) predicted secondary structure, (d) Ramachandran plot.

Molecular Docking

Twenty-one ligands were docked in the PyRx software against the proteins CDK2, c-KiT, and BRAF for this docking study. The conformation with the lowest binding affinity and zero RMSD was selected as the compound's optimal docking orientation upon the docking. Once the docking was completed the RMSD as well as binding affinity were recorded. Out of all the twenty-one phytocompounds, the common phytocompounds for all the three target proteins that had lower binding affinity than 7 and above were selected which is mentioned in Table 2 along with these 8 phytocompounds. The standard drugs were also docked with each protein and their binding affinity was recorded as shown in Table 2.

Visualization

Amongst the eight phytocompounds, the top three compounds were selected for each protein and were subjected to visualization which is done in BIOVIA Discovery Studio Visualizer. The 3D models of interaction and information about the category and type of interaction along with the bond for the corresponding amino acid residues in the ligand were also obtained.

Molecular Docking Interaction with 5UGB

The 3D interaction of 5UGB with the top three ligands that is vindolininol, lochnericine, and lochnerinine are visualized. Vindolininol has formed a conventional hydrogen bond with Leu747 with a bond distance of 2,97797 respectively seen in Figure 5(a). Lochnericine is bound to protein by a carbon-hydrogen bond with Leu747, and Ala755 with a bond distance of 3.5846 and 3.4542 respectively shown in Figure 5(b). Lochnerinine interaction with protein Glu762 and Thr785 is bounded by conventional hydrogen bonds with the distance of 3.5243 and 2.599991 respectively shown in Figure 5(c).

Molecular Docking Interaction with 2QGT

The 3D interaction of 2QGT with the top three ligands that is vindolininol, lochnericine, and lochnerinine are visualized. Vindolininol has formed a conventional hydrogen bond with Glu353 and Pro325 with a bond distance of 3.22815 and 3.48385 respectively seen in Figure 6(a). Lochnericine is not bound to protein by carbon-hydrogen bonds respectively shown in Figure 6(b). Lochnerinine interaction with protein Glu323 and Glu353 is bounded by conventional hydrogen bonds with the distance of 3.67881 and 2.85156 respectively shown in Figure 6(c).

Molecular Docking Interaction with 4OYS

The 3D interaction of 4OYS with the top three ligands that is vindolininol, lochnericine, and lochnerinine are visualized. Vindolininol has formed a conventional hydrogen bond with Tyr670 with a bond distance of 3.05174 respectively seen in Figure 7(a). Lochnericine is bound to protein Ile685

Table 2. The binding affinity of common top 8 phytocompounds and standard drugs towards all 3-target proteins.

Ligand name	5UGB	2QGT	4OYS
Vindolininol	-12.2	-10.5	-12.1
Lochnericine	-11.3	-9.8	-11.7
Lochnerinine	-11.2	-9.5	-11.6
Alpha-Amyrenyl acetate	-11.2	-9.5	-10.9
Ursolic acid	-7.7	-9.5	-8.3
Lupeol acetate	-7.2	-8.2	-8.2
Rauwolscine	-7.4	-8.1	-8.9
Ajmalicine	-7.4	-7.8	-8.9
Standard drug			
Abemaciclib	-10	-8.4	-8.1
Ribociclib	-8	-7.3	-8.8

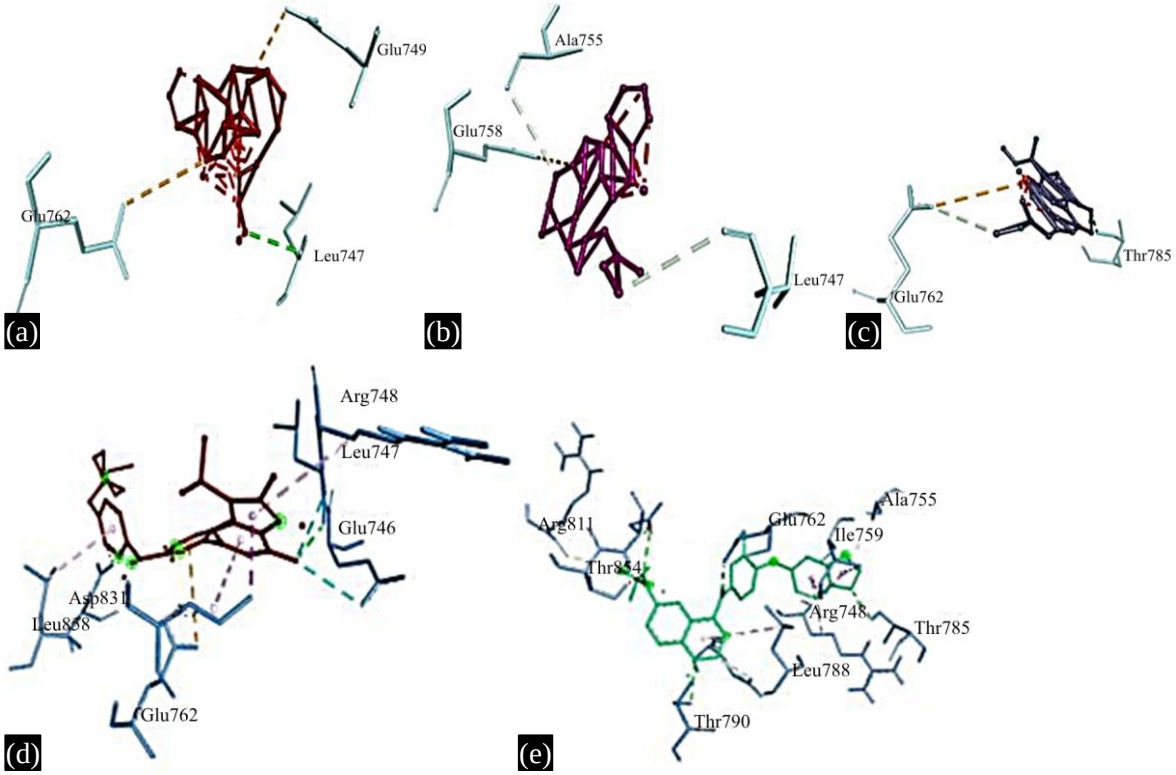


Figure 5. Protein-ligand 3D interaction of 5UGB; (a) vindolininol, (b) lochnericine, (c) lochnerinine, (d) ribociclib, and (e) abemaciclib.

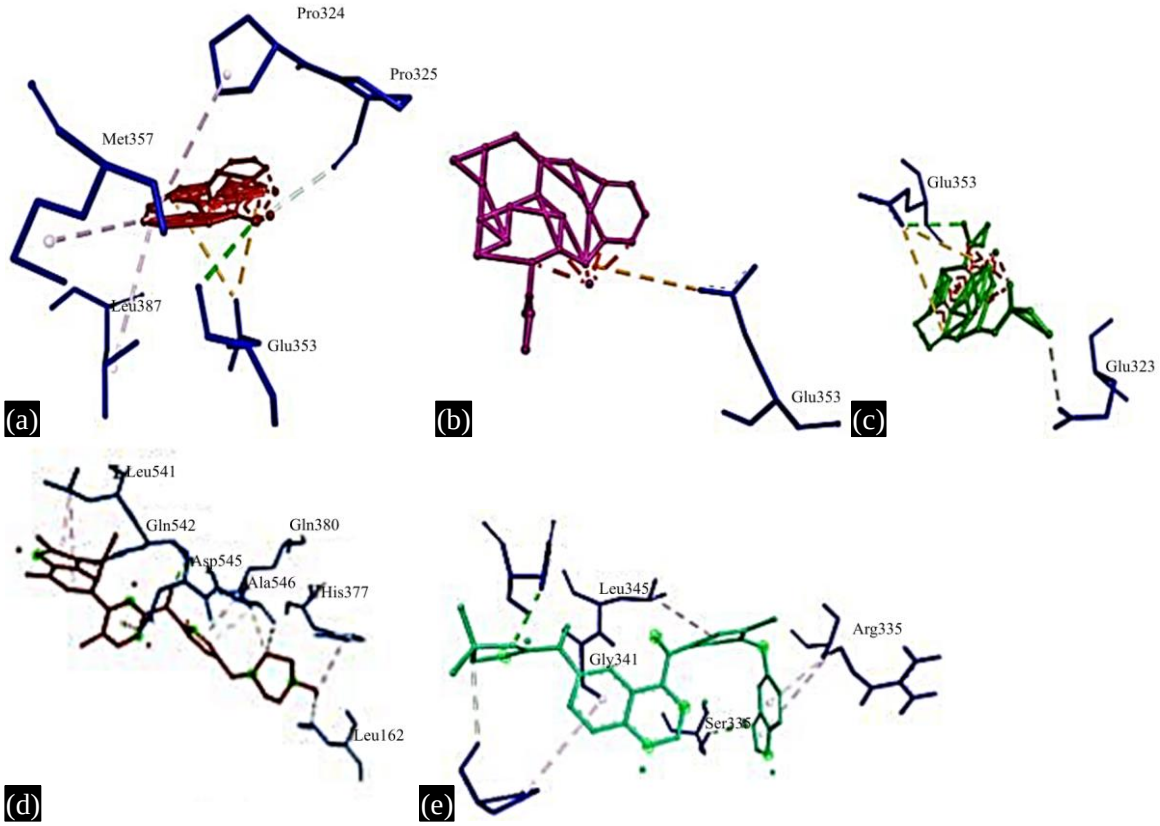


Figure 6. Protein-ligand 3D interaction of 2QGT; (a) vindolininol, (b) lochnericine, (c) lochnerinine, (d) ribociclib, and (e) abemaciclib.

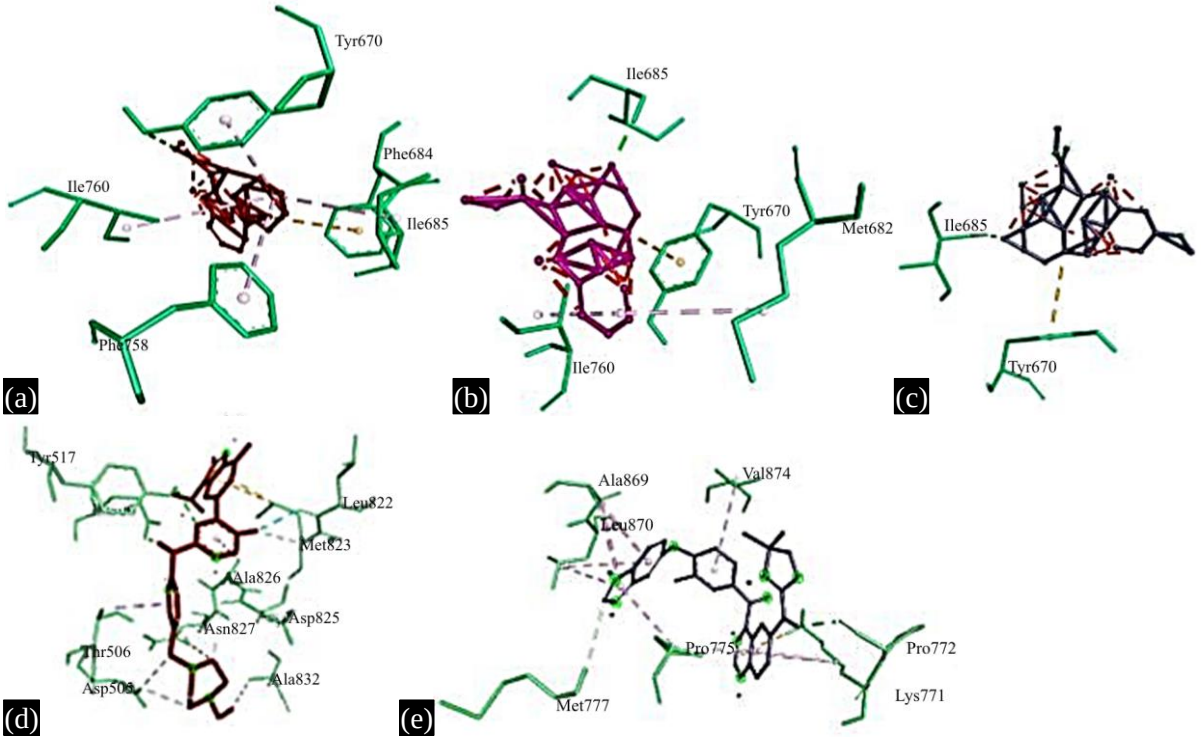


Figure 7. Protein-ligand 3D interaction of 4OYS; (a) vindolininol, (b) lochnericine, (c) lochnerinine, (d) ribociclib, and (e) abemaciclib.

and is bounded by conventional hydrogen bonds with a distance of 1.85916 shown in Figure 7(b). Lochnerinine interaction with protein Ile685 is bounded by conventional hydrogen bonds with a distance of 1.9179 as shown in Figure 7(c).

ADMET Analysis

The eight phytocompounds were selected along with their PubChem ID. These phytocompounds were examined using ADMETlab 2.0 for their physiochemical characteristics, medicinal chemistry, absorption, distribution, metabolism, excretion, and toxicity as shown in Tables 3–8.

DISCUSSION

In this study, we study breast cancer. According to earlier studies, breast cancer, a deadly form of cancer that is popularly recognized as a death sentence, is the sixth leading cause of death worldwide. In women, the prevalence of breast carcinoma is one in every nine [14]. From 1975 to 1989, the annual growth in the overall death rate from breast cancer was 0.4%, but since then it has decreased by 43% through 2020. Consequently, 460,000 breast cancer [15]. Breast cancer, being one of the high prevalent forms of cancer in women globally, is caused by DNA changes that tell your cells to proliferate out of control. It goes after breast tissue cells in this instance. Due to several risk factors in relation to bio-molecular dynamics, breast carcinogenesis is underappreciated [16]. Numerous risk factors can affect breast cancer waist-to-hip ratio, body mass index, benign breast illness, age at menstrual period, age at first premature birth, and a history of any previous breast cancer in the family were among the risk factors. Additionally, there are health issues that can be altered or maintained, such as being inactive physically. The chance of breast cancer increases, if a woman is not physically active, overweight or obese after menopause, utilizing hormone, reproductive background, or consuming alcohol [17].

The target proteins selected were EGFR, ER, and PI3KA which are responsible for causing mammary cancer. One of the first and most important targets for innovative anticancer medicines is the EGFR. About 50% of patients with inflammatory breast cancer (IBC) and triple-negative breast cancer (TNBC)

Table 3. Medicinal chemistry of top 8 ligands.

Ligands	QED	SYNTH	Fsp3	Lipinski Rule	Pain Alert
Vindolininol	0.782	6.42	0.6	Accepted	0
Lochnericine	0.606	5.271	0.619	Accepted	0
Lochnerinine	0.594	5.296	0.636	Accepted	0
Ursolic acid	0.414	4.723	0.9	Accepted	0
Lupeol acetate	0.299	4.692	0.906	Accepted	0
Rauwolscine	0.773	3.875	0.571	Accepted	0
Ajmalicine	0.801	3.936	0.476	Accepted	0
Alpha-Amyrenyl acetate	0.284	4.721	0.906	Accepted	0

Table 4. Physiochemical properties of top 8 ligands.

Ligand	MW	Vol	nHD	nHA	nRot	nRing	nHet	fChar	Flex	TPSA	LogS
Vindolininol	308.19	323.375	2	3	1	18	3	0	0.04	35.5	-3.405
Lochnericine	352.18	355.615	0	5	3	19	5	0	0.115	54.43	-3.747
Lochnerinine	382.19	381.702	0	6	4	19	6	0	0.154	63.66	-3.935
Ursolic acid	456.36	505.751	2	3	1	22	3	0	0.037	57.53	-4.383
Alpha-Amyrenyl acetate	468.4	531.553	0	2	2	22	2	0	0.074	26.3	-7.008
Lupeol acetate	468.4	531.553	0	2	3	21	2	0	0.111	26.3	-6.983
Rauwolscine	354.19	364.172	2	5	2	21	5	0	0.077	65.56	-2.88
Ajmalicine	352.18	361.535	1	5	6	21	5	0	0.077	54.56	-3.287

MW: Molecular weight, nHA: number of hydrogen acceptors, nHD: number of hydrogen bond donors, nRot: number of rotatable bonds, nRing: number of rings, nHet: number of heteroatoms, fChar: formal charge, Flex: flexibility, TPSA: topological polar surface area, LogS: logarithm of aqueous solubility value, PAINS: Pan Assay Interference Compounds.

Table 5. Absorption of top 8 ligands.

Ligand	CaCo-2	MDCK	PgP-inh	Pgp-sub	HIA	F (20%)
Vindolininol	-4.698	2.35E-05	0.005	0.361	0.013	0.005
Lochnericine	-5.02	2.87E-05	0.698	0.463	0.004	0.04
Lochnerinine	-5.094	2.48E-05	0.945	0.301	0.004	0.013
Ursolic acid	-5.221	1.39E-05	0.001	0.000	0.011	0.007
Lupeol acetate	-4.949	8.57E-06	0.43	0.000	0.009	0.037
Rauwolscine	-4.967	1.31E-05	0.006	0.274	0.009	0.02
Ajmalicine	-4.856	1.41E-05	0.017	0.001	0.006	0.006
Alpha-Amyrenyl acetate	-4.924	7.58E-06	0.409	0.000	0.008	0.093

MDCK: Madin-Darby Canine Kidney Cells, PgP-inh: P-glycoprotein inhibitor, PgP-sub: PgP Substrates, HIA: Human Intestinal absorption, F (20%): Human Oral Bioavailability 20%

Table 6. Distribution of top 8 ligands

Ligands	BBB	PBB	VDss	FU
Vindolininol	0.987	56.12%	0.864	47.14%
Lochnericine	0.984	47.69%	2.666	57.39%
Lochnerinine	0.973	64.40%	2.348	43.11%
Ursolic acid	0.719	98.84%	0.815	2.24%
Lupeol acetate	0.739	99.86%	1.767	2.09%
Rauwolscine	0.875	53.98%	2.274	40.11%
Ajmalicine	0.993	85.50%	2.153	11.40%
Alpha-Amyrenyl acetate	0.507	100.39%	1.873	1.78%

BBB: Blood Brain Barrier, PBB: Plasma Protein Binding, VDss: Volume Distribution, FU: Fraction unbound in plasma.

Table7. Metabolism and excretion of top 8 ligands.

Ligand	CYP1A2-Sub	CYP1A2-Inh	CYP3A4-Inh	CYP3A4-Sub	CL
Vindolininol	0.737	0.069	0.788	0.589	12.725
Lochnericine	0.677	0.04	0.637	0.844	9.012
Lochnerinine	0.832	0.038	0.806	0.858	9.567
Ursolic acid	0.434	0.008	0.137	0.404	3.671
Lupeol acetate	0.406	0.021	0.161	0.64	7.779
Rauwolscine	0.941	0.156	0.082	0.875	9.275
Ajmalicine	0.943	0.668	0.863	0.914	4.723
Alpha-Amyrenyl acetate	0.307	0.022	0.211	0.771	8.318

Table 8. Toxicity of top 8 ligands.

Ligands	HERG	H-HT	DILI	Ames	Carcinogenicity	respiratory	IGC50	Lc50
Vindolininol	0.032	0.091	0.01	0.153	0.019	0.788	2.777	3.468
Lochnericine	0.051	0.265	0.077	0.132	0.773	0.931	3.684	4.225
Lochnerinine	0.166	0.342	0.119	0.063	0.832	0.954	4.073	4.734
Ursolic acid	0.001	0.206	0.011	0.012	0.855	0.968	5.09	6.041
Lupeol acetate	0.028	0.232	0.087	0.015	0.031	0.688	5.543	6.985
Rauwolscine	0.662	0.461	0.066	0.027	0.009	0.97	3.077	4.056
Ajmalicine	0.911	0.6	0.07	0.754	0.211	0.976	2.7	4.274
Alpha-Amyrenyl acetate	0.002	0.17	0.053	0.012	0.019	0.955	5.5	6.921

HER: Human Ether-a-go-go related gene, H-HT: Human Hepatotoxicity, DILI: Drug-induced liver injury, IGC50: 50% growth inhibition, LC50%: 50% death.

exhibit overexpression of the EGFR gene. It has been proposed that EGFR has a significant impact on TNBC. Numerous TNBC cell lines, including MDA-MB-468 and MDA-MB-231, exhibit significant levels of EGFR expression [18]. AnxA6 expression and cholesterol buildup are induced by the extended use of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) in AnxA6-low TNBC cells, suggesting a novel mechanism of resistance [19]. Recent research has revealed that EGFR and its downstream pathway control tumor invasion, migration, and epithelial-mesenchymal transition, and that high EGFR expression is a standalone indicator of poor prognosis in IBC. ER is most individual breast cancers that begin out as estrogen-dependent, and estrogen signaling and the ER are linked to breast cancer progression. The binding of estrogen to either of the functionally and structurally different ERs, ER or ER, mediates its biological effects. We discuss the role of ER signaling in the progression of breast cancer to metastasis and the potential for targeting ER signaling interferences with cytosolic kinases as a significant extra potential treatment for treating or preventing ER-positive metastatic tumors considering mounting evidence that ER signaling would be complex, that included co-regulatory proteins as well as extranuclear actions. The major therapies for ER-positive breast cancer involve the administration of specialized estrogen receptor stimulators or chemical reactions to occur or the suppression of ER activity by lowering estrogen levels [20].

The A class of enzymes known as lipid kinases called phosphoinositol-3-kinase a (PI3Ka) is involved in the regulation of cellular processes such as angiogenesis, differentiation, survival, apoptosis, motility, cell proliferation, and DNA repair [21, 22]. These enzymes are well known for their signaling properties in the PI3K/AKT/mTOR signaling pathway, which is frequently mutated in advanced ER+ HER2 breast cancers—30% of which contain the activating PIK3CA mutation [22, 23]. Poorly regulated kinase activity as well as malignant transformation come from defects in PI3Ka pathways caused by genetic changes. One of the main reasons cancer cells develop resistance to antitumor treatments is the stimulation of this pathway, which is involved in the control of immune response, motility, growth, proliferation, and survival [21]. Mechanisms of the phosphatidylinositol triphosphate kinase (PI3K) signaling pathway contribute to angiogenesis, cell survival, proliferation, and motility, all of which are

crucial components of carcinogenesis. Due to this, many pharmaceutical firms and academic research facilities are working diligently to create inhibitors that specifically target PI3K and other crucial pathway elements [24, 21]. So, this study suggests that targeting these three proteins by their potential inhibitors like phytocompounds of periwinkle may help in treating breast cancer [25].

As observed in the result vindolininol, lochnericine, and lochnerinine are the three phytocompounds that have the best binding affinity with all three target proteins. The phytocompounds are extracted from periwinkle which has proven to have anticancer activity in various studies. Vindolininol and lochnericine are the two phytocompounds that are thought to have anticarcinogenic properties. Vindolininol is an antioxidant that may have a role in cancer prevention. Evidence suggests that attaching to tubulin prevents microtubule formation. By interfering with microtubule construction, correct mitotic spindle and kinetochore formation, and the dissociation of chromosomes throughout anaphase of mitosis, vinblastine therapy specifically causes cell cycle arrest in the M phase. stop the mitotic spindle from growing. These substances prevent the DNA-dependent ribonucleic acid polymerase from functioning in tumor cells by inhibiting the DNA synthesis and RNA synthesis pathways. According to the information it is among the best therapies for metastatic breast cancer, and its effectiveness is inversely correlated with dosage amplitude. were concerned for severe NCI-CTC-graded hematologic and nonhematologic effects other than alopecia and anemia [26, 23]. Other phytochemicals in the study also demonstrated anticancer effects, but vindolininol and lochnericine had the strongest results.

The phytocompound studies were compared with the drugs ribociclib and abemaciclib which is an FDA-approved drug. Breast cancer that has spread to other areas of your body is treated with ribociclib. The class of medications known as antineoplastics includes the medicine ribociclib. Cancer cells are eventually eliminated because it prevents their proliferation. It is indicated for the treatment of patients with metastatic or locally advanced breast cancer who also have hormone receptor (HR) positive, HER2 negative disease in combined application with either an aromatase suppressor or fulvestrant as preliminary endocrine-based therapy or even in patients who have already received endocrine therapy. Ribociclib is indeed a targeted cyclin-dependent kinase inhibitor, a category of medications that works by preventing the activity of two proteins termed cyclin-dependent kinase four and six (CDK4/6) that can reduce the growth of cancer. Overactivation of these proteins can cause cancer cells to proliferate and proliferate too quickly, which targets the CDK4 or CDK6 with either one with more accuracy [27]. The abemaciclib belongs to a class of drugs known as kinase inhibitors. It is an anticancer agent with a dual blocker of CDK4 and CDK6, which are engaged in the cell cycle and, in the event of uncontrolled activity, promote the proliferation of cancer cells. to handle a specific kind of early, hormone receptor-positive breast cancer. Those who have already received chemotherapy and antiestrogen drugs may have advanced breast carcinoma as well as breast cancer that has migrated to other organs.

According to the findings, the top three phytochemicals for each protein had lower binding affinities than the drugs, which means that they have a more effective inhibitory effect on the target protein than the chosen drug. Therefore, phytocompounds vindolininol, lochnericine, and lochnerinine in the plant periwinkle can be further studied *in vitro* and *in vivo* to create novel breast cancer inhibitors although alpha-Amyrenyl acetate, lupeol acid, and ursolic acetate had lower binding affinities than the drug there result in ADMET analysis was poor while the vindolininol, lochnericine, and lochnerinine phytocompounds showed good result in ADMET analysis.

CONCLUSION

The results of this study show that the ligand vindolininol (-10.5) has the best binding affinity with the protein ER, followed by PI3KA (-12.1) and EGFR (-12.2). For protein lochnericine (-9.8) has the best binding affinity with the protein ER, followed by PI3KA (-11.7) and EGFR (-11.3). For protein, lochnerinine (-9.5) has the best binding affinity with the protein ER, followed by PI3KA (-11.6) and EGFR (-11.2). The greatest binding affinity for the proteins is vindolininol (-12.2). According to the findings vindolininol, lochnericine, and lochnerinine are the common ligands that bind to all three

proteins effectively. Furthermore, the two common drugs ribociclib and abemaciclib were used to compare the binding affinity of these ligands. The binding affinities of these two drugs were higher than that of ligands, indicating that the phytocompounds of periwinkle selected have more potent cancer inhibitory effects on EGFR, ER, and PI3KA.

Also, the common top 8 ligands were run through ADMET analysis, in which all the ligands were examined for their physiochemical properties. The results obtained tell us that these ligands could potentially be used to treat breast cancer in future approaches for studying the urge ligands *in vitro* and *in vivo* analysis to create novel breast cancer inhibitors.

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Abbreviation

BC	:	Breast Cancer
RTK	:	Receptor tyrosine kinase
EGFR	:	Epidermal growth factor receptor
HB-EGF	:	Heparin-binding growth factor growth factor
TGF	:	Transforming growth factor
FDA	:	Food and Drug Administration
ADMET	:	Chemical absorption, distribution, metabolism, excretion, and toxicity
RMSD	:	Root Mean Square Deviation
IBC	:	Inflammatory breast cancer
TNBC	:	Triple-negative breast cancer

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