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Research Article

Ligand Based Multi-Targeted Molecular Docking Analysis of Terpenoid Phytoconstituents as Potential Chemotherapeutic Agents Against Breast Cancer: An *In Silico* Approach

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ABSTRACT

Breast cancer is one of the most common cancers in women all around the world and is a dominant cause of deaths occurring all around the globe. The available potent drugs for breast cancer show adverse effects and resistance and are found to be ineffective in patients. The high cost of currently available cancer therapy and certain limitations of current treatment make it necessary to search for novel, cost-effective and efficient methods of cancer treatment. Phytochemicals are directly involved in treatment or precursors to synthesize useful drugs. Therefore, in the current investigation, 500 terpenoid phytoconstituents and five proteins associated with breast cancer including EGFR, ERα, HER2, NF-κB and Topo IIa were selected from various databases. Selected compounds were screened for their molecular properties based on Lipinski's rule of five resulting in 235 compounds exclusion. Drug-likeness and PAINS alert properties were predicted using pkCSM and SwissADME web servers which led to the omission of 43 compounds. The remaining 222 compounds were screened to predict their ADMET properties and based on these results, 117 compounds were selected to predict the anti-breast cancer potential. Finally, 73 compounds, which showed anti-breast cancer activity prediction, were virtually screened and the top four best-scoring compounds were selected as lead-like molecules and docked with the five respective breast cancer targets. The results showed that the top four lead-like molecules exhibited greater binding affinity and lesser toxicity than the standard drugs namely 4–Hydroxytamoxifen, Daunorubicin, Erlotinib and Lapatinib.

Keywords: ADMET; Breast cancer; Chemotherapeutic agents; *In silico* analysis; Molecular docking; Terpenoids

INTRODUCTION

Cancer is one of the most hazardous, highly complex diseases resulting in abnormal proliferation, invasion and metastasis activation and uncontrollable replication. Breast cancer is the primary cause of cancer death among women and the second most frequent cancer overall after lung cancer. International Agency for Research on Cancer (IARC) of World Health Organization (WHO) reported 19.29 million new cases of cancer across all ages and genders in 2020. In India, 1,78,361 new breast cancer cases and 90,408 deaths were reported in GLOBOCAN, 2020. In 2022, around 19.3 million cancer incidence cases and 10 million cancer mortality cases were reported globally. An estimated number of 3,00,590 incident cases and 43,700 deaths globally is reported in Cancer Statistics, 2023. There are some clinical trials in place for specific types of breast cancer prevention. [1–4]

Breast cancer can occur due to hereditary or non-genetic factors. The genes for breast cancer-1 (BRCA1) and breast cancer-2 (BRCA2) are important in the growth of breast cancer. The genes for checkpoint kinase 2 (CHEK2) and tumour protein p53 (TP53) are also linked to the emergence of breast cancer. Various signalling pathways are dysregulated in cancer cells, according to the literature. Breast cancer cells with reduced levels of a non-receptor tyrosine kinase such as breast tumor kinase (BRK) experienced reduced activation of EGFR (epidermal growth factor receptor) controlled signaling proteins. [5]

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Among all cancer types, breast cancer continues to rank as one of the most dangerous diseases, accounting for a large number of cancer-related deaths among women globally and also the risks are rising in men affected by breast cancer. Additionally, the number of Breast cancer diagnoses is rising annually. Breast cancer was treated for a very long time with hormone therapy, surgery, chemotherapy and radiotherapy. The multidrug resistance and severe adverse effects of current therapeutic methods, however, are making them increasingly ineffective. In order to reduce the aggressiveness of breast cancer and to prevent cancer cell multiplication, invasion and metastasis, it is urgently necessary to create more effective and safe treatments.[6–9]

Cancer cells possess the ability to self-renew and can develop all types of cells. Hemostasis, embryogenesis, and proper development are all largely regulated by several signaling cascades. Various studies demonstrated that inhibitors (natural or synthetic) reduced drug resistance in cancer cells *in vitro* by inducing apoptosis and inhibiting cell cycle migration and proliferation. The studies revealed that the various phytochemicals especially, terpenoids play an important role in preventing activity against the proliferation of breast cancer cells and suppressing of *in vitro* proliferation process.[10–14]

Terpenoids are one of the largest classes of secondary metabolites obtained from natural resources and are structurally composed of isoprenoid units. Terpenoids are classified into six classes, including monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes, based on their structural characteristics. Among the numerous biological applications, terpenoids are extremely applied in antitumour activities including anti-angiogenic, apoptotic and anti-metastatic anti-proliferative activities.[15,16] Terpenoids are primarily employed as novel anti-cancer agents in various pharmaceutical industries.For instance, the natural anti-cancer compound namely, elemene suppresses brain tumors, lung cancer, liver cancer, nasopharyngeal carcinoma and other cancers. Terpenoids found to have an inhibitory effect on cell proliferation and tumor growth in a variety of human cancers, according to *in vitro* and *in vivo* investigations. Some terpenoids induce their anticancer effect by triggering different stages of cancer growth, such as reducing the early stage of carcinogenesis by inducing cell cycle arrest, preventing cancer cell differentiation and activating apoptosis, to produce an anticancer effect. Recent research has focused on understanding the ”terpenoid-induced autophagy” phenomenon in cancer cells in both *in vitro* and *in vivo* settings.[17,18] The need for steroidal terpenoids for treating breast cancer is that the steroidal terpenoids can exhibit potential anticancer activity by triggering the various stages of cancer progression and development. It has the ability to suppress cancer development at the early stage through cell arresting and induces apoptosis. For example, steroidal terpenoids like Salvinorin A, cannabinoids, ginkgolide, bilobalide, menthol and etc had been reported for exhibiting excellent anticancer activity. Since breast cancer is hormone-related cancer, the present study aims to identify lead-like molecules belonging to di- and tri-terpenoids that collectively come under steroidal terpenoids using a ligand-based drug discovery approach.

MATERIALS AND METHODS

Ligand preparation

The list of 500 steroidal terpenoids were collected from the Terokit database (http://terokit.qmclab.com/index.html).[19] Structures of these compounds as 3D sdf (.sdf) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). These structures were exported to ChemDraw Ultra 12.0 (www.cambridgesoft.com) for energy minimization and geometry correction. The outputs of the structures were saved in mol file (.mol) and pdb (.pdb) formats which were used for further *in silico* studies.

Protein preparation

Crystal three-dimensional structures of Epidermal Growth Factor Receptor tyrosine kinase domain (PDB ID: 1M17), Human Estrogen Receptor Alpha (PDB ID: 3ERT), Human HER2 kinase domain (PDB ID: 3PP0), Human IkB kinase beta (PDB ID: 4KIK) and Human Topo IIa ATPase/AMP-PNP (PDB ID: 1ZXM) were downloaded from RCSB Protein Data Bank (http://rscb.org) in the pdb format. The stereochemical properties of each target protein were assessed based on the information obtained from PDB X-Ray structure Validation Report for each PDB structure. The resolution and R-values showed the goodness of the protein model being used. The X-ray crystal structure with resolution values of 2.0Å or less and R-values of 0.2 or less is considered acceptable. Moreover, the structures of the proteins were evaluated based on the Ramachandran plot using Molprobity (http://molprobity.biochem.duke.edu/).[20] Proteins were energy minimized, water molecules were removed and hydrogen and charges were added. Finally, 3D structures of the target proteins were saved and used for virtual screening and docking studies.

Molecular properties and toxicity predictions

The phytochemicals were screened for their molecular properties and drug-likeness using the SwissADME web server (http://www.swissadme.ch/). Toxicity was predicted by using the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm/prediction) and ProTox II web server (https://tox-new.charite.de/protox_II/). Cytotoxicity activity against breast cancer was predicted by using the CLC-prediction web server (http://www.way2drug.com/Cell-line/index.php

cancer).

Virtual screening

Virtual screening was performed using iGEMDOCK software version 2.1 (http://gemdock.life.nctu.edu.tw/dock/igemdock.php) with the following parameters: standard flexible docking option with the population size of 200, 70 generations and 2 solutions.[21] The binding site of the target was 8Å. The empirical screening function of iGEMDOCK was estimated as: Fitness = vdw + Hbond + Elec.

Molecular docking and visualization

Molecular docking was performed using AutoDock 4.0 (http://autodock.scripps.edu/) with the Lamarckian genetic algorithm. The docking parameters selected were 200 docking runs and a population size of 200. The binding energy of the protein coordinate was evaluated by a three-dimensional grid box of 40x40x40 (Number of grid points in xyz) created with a spacing of 0.375Å.[22] Biovia Discovery Studio version 21.1.0 (https://www.3dsbiovia.com/) was used to visualize the docking results.

Validation

The docking energies of the phytochemicals were compared with the commercially available FDA-approved antitumour drugs namely 4-Hydroxytamoxifen, Daunorubicin, Erlotinib and Lapatinib for the validation of results. The docking energy for a given protein-ligand pair comprised of the intermolecular interaction energies including internal steric energy, hydrogen bond interaction energy, Van der Waals force and columbic electrostatic energy of the phytochemical. The lowest binding energy of the protein-ligand complex has been considered to be the best.

RESULTS AND DISCUSSION

In drug discovery, many potential drugs have failed in clinical studies or late drug discovery processes due to poor drug-like properties and adverse effects. An important phase in the process of discovering new drugs is the early prediction of various properties of molecules. To make better decisions before initiating experimentation on lead compounds, numerous computational tools are being applied throughout the research areas from the academy and the pharmaceutical industry. Drug discovery and development are expensive and time-consuming processes. All the developed molecules in the current inquiry were put through *in silico* analysis to check for toxicity risks and drug-relevant features, which are crucial components in determining the drug-like properties of the lead molecules.[23] In the present work, a total of 500 compounds belonging to steroidal terpenoids based on molecular weight (<500 Da), were selected from the TeroKit database (Table containing compound names along with their CID numbers are given in the supplementary file S1).

Based on the Lipinski's rule of five, molecular properties and drug-likeness of the 500 terpenoid phytochemicals and the standards were evaluated. To function and operate effectively in the biological system, a potential drug substance must be easily absorbed and distributed. These molecular and drug-likeness parameters are employed to determine the drug-like nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery. According to this tool, if the TPSA is $\leq$ 140Å^2, the molecule will appear to be polar, be well absorbed by the GI tract when given orally, and be more likely to cross the BBB and enter the CNS. If the molecule has a high logP (lipophilicity) value (>0), it is more likely to be oxidized by the cytochrome CYP450 and to be heavily attached to plasma proteins.[24,25] Water solubility is one of the most important key factors which alter the pharmacokinetic profile of a molecule. If a molecule is poorly soluble, then it may lead to potential problems for dosage formulation and a small amount of the administered molecule is excreted by the kidney. Total Polar Surface Area (TPSA), is a crucial parameter used to determine how permeable a membrane is by comparing the surfaces of polar atoms in molecules. Molecules with a molecular mass of less than 500 g/mol fall into the TPSA range of 0-140 Å^2. It is reported that the compound possesses better absorption and increased sensitivity to cross BBB (Blood Brain Barrier) if the TPSA value is within these previously indicated ranges.[26,27] The 235 compounds showed violations to these parameters and were omitted for further screening. Based on the filter process, 265 compounds were selected for drug-likeness screening and pains (Pan Assay Interference Structures) alert determination using SwissADME webserver. Around 43 compounds showed violations towards drug-likeness and Pains alert, and they were omitted for further screening and the compounds with no violations are considered to be suitable for oral administration.

Based on the filter process, a total of 222 compounds were screened for their pharmacokinetic parameters such as absorption, distribution, metabolism and elimination using pkCSM online web server. The Caco-2 cell line is made up of human epithelial colorectal adenocarcinoma cells. The Caco-2 monolayer of cells is widely used as an *in vitro* tool for the human intestinal mucosa to evaluate the absorption of orally administered medications.[27] If a molecule has a predictive value greater than 0.9, it is thought to have a high Caco-2 permeability. The intestine is the most essential primary site for the absorption of a drug from an orally administered solution. If the absorption of a molecule is less than 30%, then it is considered to be poorly absorbed.

The volume of distribution (VDss) is the theoretical volume over which a total dose of a drug must be distributed uniformly to achieve the same concentration as blood plasma. It can be easily affected by renal failure and

dehydration. If VD is higher, then the drug distributed to tissue is higher than plasma.[28] VDss is considered low if the value is below 0.71 L/kg (log VDss < -0.15) and high if above 2.81 L/kg (log VDss > 0.45).

Blood Brain Barrier (BBB) guards brain from the exogenous substances. The ability of a drug to enter the brain is an essential parameter in reducing the side effects and toxicities or improving the efficacy of drugs whose pharmacological activity is within the brain. The logarithmic ratio of brain to plasma drug concentrations, or log BB, is used to quantify blood-brain barrier permeability in living animal models. Molecules having a log BB value of greater than 0.3 are thought to easily penetrate the blood-brain barrier, while those with a log BB value of less than -1 are thought to be poorly dispersed to the brain. The blood-brain permeability-surface area product (logPS) is a more precise measurement and is derived via *in situ* brain perfusions in which the substance is administered directly into the carotid artery. There are no systemic distribution effects that could affect brain penetration. It is considered that substances with a logPS larger than -2 can enter the central nervous system (CNS), but substances with a logPS lower than -3 are considered to be unable to penetrate to CNS (He et al., 2018).[29]

Cytochrome P450 is an essential enzyme used for detoxification of the body which is mainly found in the liver. It metabolizes and facilitates the excretion of xenobiotics. The cytochrome P450s can both activate and deactivate many drugs. Drug metabolism may be impacted by inhibitors or substrates of this enzyme, which is not recommended. Therefore, it is essential to evaluate the capacity of the drug to inhibit the different isoforms of this enzyme as well as its potential to act as a substrate.

The proportionality constant Drug clearance is essentially a combination of hepatic (liver metabolism and biliary clearance) and renal clearance, calculated using CLtot. It is necessary to calculate the dose rates to reach steady state concentrations because it is closely related to bioavailability.[29] The predicted total clearance of the phytochemicals is given in log (ml/min/kg). The renal uptake transporter known as renal Organic Cation Transporter 2 (OCT2) is essential in the renal clearance of medications and endogenous chemicals. OCT2 substrates and concurrently given OCT2 inhibitors may interact negatively. Analyzing a molecule's ability for OCT2 transport can reveal important details about its clearance as well as any potential contraindications.

An important phase in the process of developing a medicine is predicting the toxicity of chemicals. In addition to being faster than employing animals to determine harmful or dangerous levels, computational toxicity predictions can also help to reduce the total number of animal experiments. Drug-induced liver damage is a major cause of medication withdrawal and a significant safety concern for pharmaceutical research. The Ames toxicity parameter is widely used for screening the mutagenic potential of new chemicals and drugs. Ames Salmonella/microsome mutagenicity assay (Salmonella test; Ames test) is a short-term bacterial reverse mutation assay used to detect the new drugs that can cause genetic damage resulting in gene mutation. These mutations act as hot spots for mutagens to cause DNA damage via different mechanisms.[30]

The main factor causing acquired long QT syndrome and deadly ventricular arrhythmia is the inhibition of the potassium channels expressed by the hERG (human ether-go-go gene).[31] Many medicinal compounds have been removed from the pharmaceutical market as a result of hERG channel inhibition. In the present study, the compounds that were found to be hERG inhibitors are omitted and only the compounds that were not found to be hERG inhibitors were selected for further studies. A substance was deemed to be hepatotoxic if it caused at least one pathological or physiological liver event that is strongly linked to the disruption of the liver's normal function. In the present study, the 105 compounds that were considered to be hepatotoxic were omitted for further screening.

The toxicity profiles of different compounds can be compared using the fatal dosage values, a typical factor of acute toxicity. The LD50 is the concentration of a drug that, when administered to a set of test animals or microorganisms, causes a 50% inhibition or survival rate. In the present study, around 105 compounds that showed greater toxicity were omitted and only the remaining 117 compounds were subjected to predict their anti-breast cancer activity using the CLC-Pred online web server. After the screening of anti-breast cancer activity, finally, 73 compounds were selected for virtual screening against various cancer targets using iGEMDOCK software. In the present study, the five different anti-breast cancer targets were identified from the literature namely, Epidermal Growth Factor Receptor tyrosine kinase domain (1M17), Human Estrogen Receptor Alpha (3ERT), Human HER2 kinase domain (3PP0), Human IkB kinase beta (4KIK) and Human Topo IIa ATPase/AMP-PNP (1ZXM), which were validated by a Ramachandran plot that showed more than 95% favourable region which is valid for drug screening. The experimental data and validation details of the protein are given in Table 1 and the protein structures and Ramachandran plot of selected 5 breast cancer targets are given in Figure 1. Further to confirm the results obtained from virtual screening, the top 4 compounds and standards were docked with five different anti-breast cancer targets using AutoDock 4.2 Software.

Based on the binding score and mode of interaction, top four molecules from each target were identified as lead-like molecules against breast cancer including 2, 3-Dihydrowithaferin A **(298),** (3Ar,3bS,5aS,6R,8aS,8bS,10R,10aS)-10-hydroxy-6-[(1S)-1-[(2R)-5-(hydroxymethyl)-4-methyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-3a,5a-dimethyl-3-oxo-

Table 1: Selected Breast Cancer Targets and their Experimental Data

PDB ID	Resolution (Å)	Refinement	R-value	Favoured Regions (%)	Ramachandran outliers (%)
1M17	2.6	X-PLOR 98.1	0.251	89.2	7
3ERT	1.9	X-PLOR 3.854	0.229	96.5	4
3PPO	2.25	REFMAC 5.5.0109	0.185	95.6	2
4KIK	2.83	BUSTER 2.11.5	0.186	97.3	3
1ZXM	1.87	CNS 1.1	0.22	96.6	5

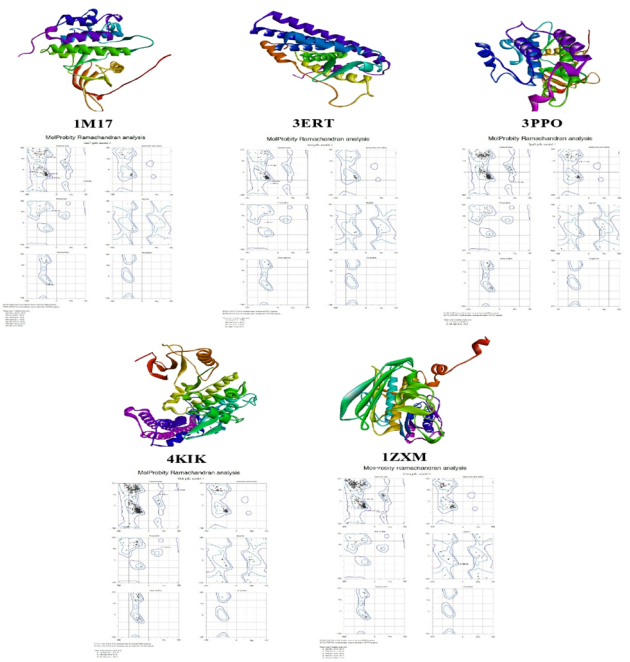

Fig. 1: Protein structures and Ramachandran plot of selected Breast Cancer targets used for the present study

1,2,3b,4,5,6,7,8,8a,8b,9,10-dodecahydroindeno[6,7-e]indene-10a-carbaldehyde **(229),** Jaborosalactone B **(81),** Jaborosalactone O **(156),** 8alpha,9alpha-Epoxy-4,4,14alpha-trimethyl-3,7,11,15,20-pentaoxo-5alpha-pregnane **(7),** Antiarotoxinin A **(485),** (1S,2S,4S,6S,7S,8R,9S,12S,13R)-6-[(2R,3R,4R)-3,4-Dimethyl-5-oxooxolan-2-yl]-6-hydroxy-7,9,13-trimethyl-5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icosa-14,17-dien-16-one **(167)**, Bendigole D **(60)**, Jaborosalactone B **(81)**, Certonardosterol G **(372)**, (23R,24S)-Chiograsterol A **(349)**, Jaborosalactone A **(305)**, Withacoagulin **(76)**, (1S,2R,6S,7R,9R,11S,12S,15R,16S)-15-[(1S)-1-[(2R)-4,5-Dimethyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-6-hydroxy-2,16-dimethyl-8-oxapentacyclo[9.7.0.02,7.07,9.012,16]octadecan-3-one **(297)**, (22R)-1-Oxo-12beta,21,22,27-tetrahydroxyergosta-2,5,24-triene-26-oic acid delta-lactone **(82)**, [(3R,8R,9S,10S,13R,14R)-10,13-Dimethyl-17-oxo-1,2,3,4,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-yl] hydrogen sulphate **(262)**, Androsterone sulphate **(261)**, 7alpha,12alpha-Dihydroxy-3-oxochola-1,4-dien-24-oic acid **(73)** and Murideoxycholic acid **(48)**. The binding energy in terms of kcal/mol, types of interactions such as hydrogen bond and hydrophobic along with distance is presented. The ligand in protein pocket and 2D interactions are shown in Figures 2, 3, 4, 5 and 6.

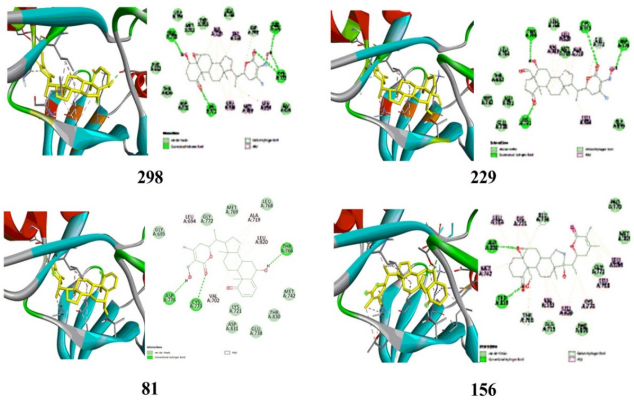

Fig. 2: Ligand in protein pocket and 2D interactions of top four Terpenoid Phytochemicals with EGFR (1M17)

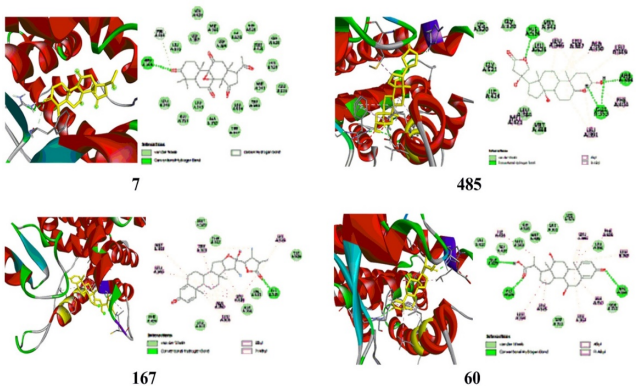

Fig. 3: Ligand in protein pocket and 2D interactions of top four Terpenoid Phytochemicals with ERα (3ERT)

Compounds **298, 229, 81** and **156** showed good interaction profiles and were identified as lead-like molecules against the EGFR target (1M17). In compound **298,** conventional hydrogen bond and carbon-hydrogen bond interactions were observed with LYS721, GLU738, GLY772, CYS773 and ASP776 residues. The complex showed hydrophobic interactions like alkyl interactions

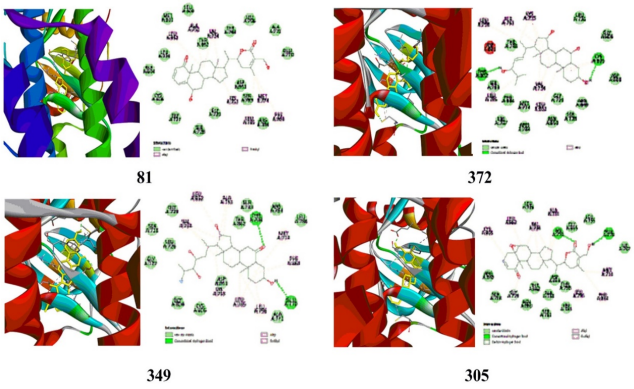

Fig. 4: Ligand in protein pocket and 2D interactions of top four Terpenoid Phytochemicals with HER2 (3PPO)

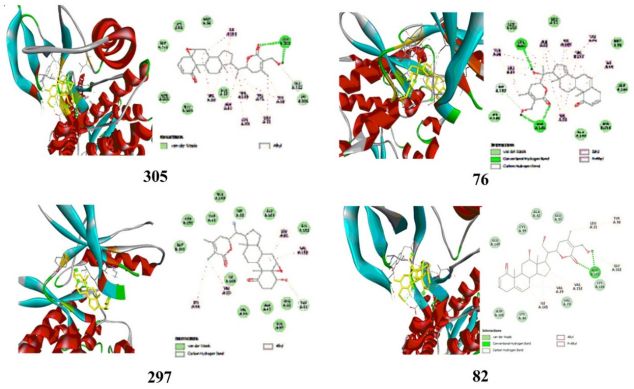

Fig. 5: Ligand in protein pocket and 2D interactions of top four Terpenoid Phytochemicals with NF-κB (4KIK)

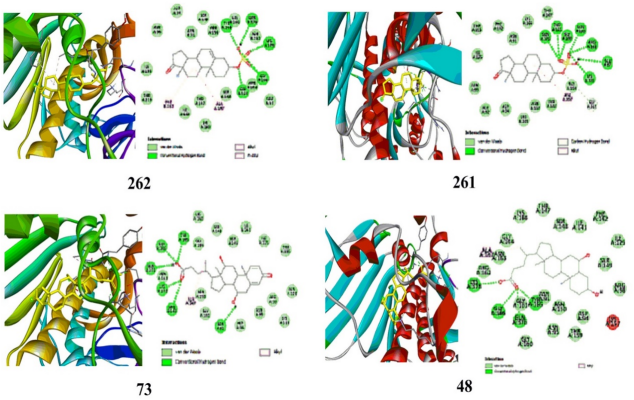

Fig. 6: Ligand in protein pocket and 2D interactions of top four Terpenoid Phytochemicals with Topo IIa (1ZXM)

with LEU694, VAL702, ALA719, MET769 and LEU820 interacting amino acid residues with the binding energy of -11.28 kcal/mol. Compound **229** with a binding energy of -10.64 kcal/mol showed hydrogen bond interactions with LYS721, THR766, GLY772, CYS773 and ASP776 residues and showed hydrophobic interactions with LEU694, VAL702, ALA719 and LEU820 interacting amino acid residues. Compound **81** was stabilized with the hydrogen and hydrophobic interactions with THR766, CYS773, ASP776, LEU694, VAL702, ALA719 and LEU820 interacting amino acid residues with the binding energy of -10.6 kcal/mol. Compound **156** with the binding energy of -10.08 kcal/mol showed hydrogen bond interactions with GLU738, THR766, THR830 and ASP831 interacting residues and hydrophobic interactions with LEU694, VAL702, LYS721, MET742, LEU764, LEU768 and CYS773 interacting residues.

Compounds **7, 485, 167** and **60** showed better binding affinity and were identified as lead-like molecules against the ERα target (3ERT). In compound **7,** hydrogen bond interactions like conventional hydrogen bond and carbon hydrogen bond interactions were observed with ARG 394 and PHE 404 with the binding energy of -10.92 kcal/mol. Compound **485** with a binding energy of -10.67 kcal/mol showed hydrogen bond interactions with GLU353, ARG394 and HIS524 residues and showed alkyl and π-alkyl interactions with LEU346, LEU349, ALA350, LEU387, LEU391, PHE404 and MET421 interacting amino acid residues. In compound **167,** conventional hydrogen bond interaction was observed with CYS530 and hydrophobic interactions with MET343, LEU346, ALA350, TRP383, LEU384, LEU525 and LYS529 interacting amino acid residues with the binding energy of -9.26 kcal/mol. Compound **60** showed hydrogen bond interactions with ARG394, GLU419 and HIS524 interacting residues and hydrophobic interactions with LEU346, LEU349, ALA350, LEU384, LEU387, PHE404, ILE424 and LEU525 interacting amino acid residues with the binding energy of -9.07 kcal/mol.

Compounds **81, 372, 349** and **305** showed greater binding affinity and were identified as lead-like molecules against the HER2 target (3PPO). In compound **81** , hydrophobic interactions like alkyl and π-alkyl interactions were observed with VAL734, ALA751, LYS753, MET774, LEU785, LEU852 and PHE864 with the binding energy of -10.5 kcal/mol. Compound **372** showed hydrogen bond interactions with CYS805 and THR862 residues and showed hydrophobic interactions with VAL734, ALA751, LYS753, LEU785, LEU796 and LEU852 interacting amino acid residues with the binding energy of -9.89 kcal/mol. In compound **349,** conventional hydrogen bond interaction was observed with GLU770 and THR798; hydrophobic interactions with VAL734, ALA751, LYS753, MET774, LEU785, LEU796, LEU852 and PHE864 interacting amino acid residues with the binding energy of -9.83 kcal/mol.

Compound **305** showed hydrogen bond interactions like conventional hydrogen bond and carbon-hydrogen bond interactions with GLY729, LYS753 and GLU770 interacting residues and hydrophobic interactions like alkyl and π-alkyl interactions with VAL734, ALA751, MET774, LEU796, CYS805, LEU852 and PHE864 interacting amino acid residues with the binding energy of -9.69 kcal/mol.

For the NF-κB target (4KIK), compounds **305, 76, 297** and **82** showed good interaction profiles and binding affinity. In compound **305 ,** hydrogen bond interactions with GLY102 and ASP103; hydrophobic interactions with LEU21, VAL29, ALA42, VAL74, TYR98, CYS99, VAL152 and ILE165 with the binding energy of -11.72 kcal/mol were observed. Compound **76** showed hydrogen bond interactions with CYS99, GLY102 and ASP103 residues and showed hydrophobic interactions with LEU21, VAL29, ALA42, LYS44, VAL74, TYR98, VAL142 and ILE165 interacting amino acid residues with the binding energy of -11.36 kcal/mol. In compound **297,** carbon-hydrogen bond interaction was observed with TYR98 and alkyl interactions with LEU21, VAL29, LYS44 and VAL152 interacting amino acid residues with the binding energy of -9.93 kcal/mol. Compound **82** showed hydrogen bond interactions like conventional hydrogen bond and carbon-hydrogen bond interactions with ASP103 and GLY102 interacting residues and hydrophobic interactions like alkyl and π-alkyl interactions with LEU21, VAL29, TYR98, VAL152 and ILE165 interacting amino acid residues with the binding energy of -9.78 kcal/mol.

For the topoisomerase (TOPO IIa-1ZXM) target, compounds **262, 261, 73** and **48** were identified as lead-like molecules. In compound **262,** hydrogen bond interactions with GLY164, TYR165, GLY166, GLN376 and LYS378; hydrophobic interactions with PHE142 and ALA167 with the binding energy of -13.07 kcal/mol. Compound **261** showed hydrogen bond interactions with GLU87, GLY161, ARG162, ASN163, TYR165 and GLY166 residues and showed hydrophobic interactions with ALA167 interacting amino acid residue with the binding energy of -11.69 kcal/mol. In compound **73,** carbon hydrogen bond interaction was observed with ASN91, ARG162, TYR165, GLY166, GLN376 and LYS378 and hydrophobic interaction with ALA167 interacting amino acid residue with the binding energy of -11.08 kcal/mol. Compound 4**8** showed hydrogen bond interactions with TYR165, GLY166, GLN376 and LYS378 interacting residues and hydrophobic interaction with ALA167 interacting amino acid residue with the binding energy of -10.84 kcal/mol.

Among 500 terpenoid phytochemicals, the top 4 compounds of each target were identified as lead-like molecules based on significant molecular, pharmacokinetic and toxicity properties. Lead-like molecules showed better binding affinity to respective five protein targets and also showed less toxicity than standard drugs. Based on the docking results, EGFR target (1M17) was found to have better binding affinity against the compound **298,** ERα target (3ERT) showed better binding energy against compound **7,** HER2 target showed better binding energy against compound **81,** NF-κB target (4KIK) showed better binding energy against compound **305** and topoisomerase (TOPO IIa-1ZXM) showed better binding energy against compound **262.** Among these five different breast cancer targets, Human Topo IIa ATPase/AMP-PNP (1ZXM) target was found to be best anti-breast cancer target, followed by topoisomerase, the Human HER2 kinase (3PP0) target was also found to be an active target. In conclusion, we propose these identified lead compounds can be extensively used as potential chemotherapeutic agents in the treatment of breast cancer.

CONCLUSION

Breast cancer is the most common type of cancer in women worldwide. Therefore, there is an urgent need for developing more potent, secure and selective inhibitors of breast cancer receptors. Since breast cancer is hormone-related cancer, the present study aims to identify lead-like molecules belonging to di- and tri-terpenoids by ligand-based drug discovery approach. The phyto-ligands were obtained from Terokit database and based on the physicochemical properties, solubility, lipophilicity, pharmacokinetic profile and toxicity, the compounds were filtered off and the compounds which shows anti-breast cancer potential was selected. The finalized compounds were virtually screened to identify hit molecules using iGEMDOCK against five different breast cancer targets such as EGFR, ERα, HER2, NF-κB and TOPO IIa. Based on the score, the top ten hit molecules from each target were selected and docked by using AutoDock 4.2 to identify lead-like molecules. Based on the binding score and mode of interaction, the top four molecules from each target were identified as lead-like molecules against breast cancer. These top four molecules showed greater binding affinity and lower toxicity than the standards. Therefore, these potent chemotherapeutic agents can be employed for the effective treatment of breast cancer. Further in vivo and in vitro studies are required to confirm the potential of these lead-like compounds.

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Conflicts of Interest

The authors declared that they have no conflict of interest.

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