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GALANGAL PHYTOCOMPOUNDS IN TUMOUR TACTICS - AN *IN SILICO* ANALYSIS TARGETING BREAST CANCER

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ABSTRACT

Current research in drug discovery from medicinal plants provides novel and important leads against cancer targets. In the present study, breast cancer proteins docked with natural compounds derived from the plant, *Alpinia galanga* (L.) Willd offers a great hope in the identification of drug targets and potential lead compounds. Lig Prep module was used to retrieve all molecules from GC-MS results and processed it to be docked with diseased protein. The drug likeliness and toxicity of the selected molecules were performed using QikProp module from Schrodinger. Glide module identifies favorable interactions between lead molecules and receptors and the best complex was analyzed through Desmond. Principal descriptors and property predictions of lead compounds were observed to be under the acceptable limit. Nortrachelogenin was best docked with KLRG1 (-7.24 Kcal/mole) and STK10 (-7.21 Kcal/mole). Alpha-terpineol was best bound to BAX (-5.42 Kcal/mole). 3-phenyl-2-butanone showed multiple binding interactions with PTEN, CHEK1, PALB2 and BRCT7. Simulation interactions revealed that Checkpoint kinase1 complexes with 3-phenyl-2-butanone as the best complex. Altogether the findings explored the unique potential of galangal phytocompounds in future cancer therapies with less undesirable side effects

KEYWORDS: *Greater galangal, Nortrachelogenin, 3-phenyl-2-butanone, Breast cancer, Schrodinger*

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INTRODUCTION

From ancient times, plants have been used as an important source of medicine due to the presence of a wide spectrum of biologically active compounds. They are considered to be perfect natural laboratories for the synthesis of various molecules ranging from simple skeleton to highly complex chemical structures[1]. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [2]. A recent report on the docking analysis of 3-O-acetyl-11-keto-β-boswellic acid, a triterpenoid obtained from aqueous root extract of *Rotula aquatica* exhibited the ability of the compound towards inhibition of calcium oxalate crystallization in urolithiatic conditions[3]. Meenambiga *et al*.[4] provides new insights into developing novel COX-2 inhibitors from *Inonotus* species, which possess potential medicinal value with anti-inflammatory properties. Previous reports of Aggarwal[5] described the process of tumor suppression of the transcription factor NF-kB by withanolides - ergostane type steroids. Etti *et al.*[6] discovered anticancer phytomolecules from *Artocarpus* species, which evoked growth inhibition of estrogen receptor signaling in breast cancer.*Alpinia galanga* (L.) Willd is one such candidate plant, needed to be investigated as a promising anticancer agent. It was reported as a gold mine for future therapeutics. It is a rhizomatous plant having a distinctive aroma, distributed widely in tropical areas and used as a medicine in many countries [7-10]. Different parts of the plant have shown to possess various bioactivities. The dried rhizome provides the drug Greater galangal[11]. The extensive use of rhizomes in the preparation of ayurvedic medicines prompted us to take up and study this plant for its anticancer potential.The recent reports on breast cancer have badly attacked women population in India. A new study conducted by International consortium of researchers, coordinated by the Institute for Health Metrics and Evaluation (IHME) at the University of Washington shows breast cancer is the top killer of women in India (The Indian Express, May 2015). ICMR has projected that India is likely to have over 17.3 lakh new cases of cancer and over 8.8 lakh deaths due to the disease by 2020 with cancers of the breast, lung and cervix topping the list. The gush in breast cancer in India mirrors the global situation. In 2013, 47,587 women died due to breast cancer. In this scenario, the biologically active compounds from medicinal plants have become an inherent acquisition to modern medicine for the better treatment of cancer. The previous report of *in silico* screening of chemical compounds from Zingiberaceae family showed that the bioactive ligands isolated from *A. galanga* were found to be the most potent inhibitor of neuraminidase. The compound, 1, 2-di-O-β-Dglucopyranosyl-4-allylbenzene (BGA) had better affinity and ability to inhibit neuraminidase[12]. Malami *et al*.[13] have recently demonstrated the applicability of molecular docking studies in discovering potential uridine cytdine kinase 2 inhibitors from the rhizomes of *A. mutica.* In another study, bioactive compounds from *A. purpurata* proved its role as novel inhibitors for CXCR4, a receptor for chemokine[14]. Also, *A. oxyphylla* exhibits anti-diarrhea effects partially by affecting proteins of NHE3 and AQP4 with its active ingredient Tectochrysin[15]. These findings support the identification of novel natural anticancer compounds from *A.galanga*, depicted as a highly demanding avenue of cancer therapies[16].A holistic approach is a system of health care which offers a cooperative relationship among phytochemical, biological and computational methods leading towards optimal health. This system improves the speed of the screening, isolation, and structure elucidation processes, as well addressing the suitability of screens for natural product extracts [17]. Among these *in silico* prediction systems are cheaper, rapid, and reproducible, have low compound synthesis and can undergo constant optimization[18]. Molecular docking is a computational method widely used to elucidate the mechanism of action and rationalize structure-activity relationships of natural products [19]. Molecular docking studies reduce the laboratory work, as well as justify chemical/physical explanations for observed phenomena. This programs employed empirical energy function to determine and optimize the interaction energy between the drug candidate and the active site[20]. Ferreira *et al*.[21] described molecular docking as an efficient bioinformatics technique used for finding out potent compounds acting against specific targets/ biomarkers of specific disease, without spending much time as like in a normal drug discovery process. Moreover *in silico* simulations can be used to propose protein-ligand binding characteristics for molecular structures, including known constituents of plant materials. Karplus and McCammon[22] reported that MD simulation depicts the dynamic behavior of a biomolecule in the actual biological scenario. In MD simulations, chemical bonds and atomic angles are modeled using simple virtual springs, and dihedral angles are modeled using a sinusoidal function. These simulations, coupled with experimental data have a significant impact on the drug discovery process[23]. Therefore compounds that perform well in *in silico* predictions can be used as promising starting materials for experimental work and can be promoted for the development of cancer therapies.The present investigation is an attempt to identify the major phytocompounds of galangal rhizomes as anti-breast cancer molecules through molecular docking and simulation approaches.

MATERIALS AND METHODS

Collection and authentication of the plant

Healthy plants of *Alpinia galanga* (L.) Willd was collected from Alappuzha, Kerala. The plant material was identified and their authenticity was confirmed by Dr. M. Palanisamy, Scientist-D, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu (BSI/SRC/5/23/2015/Tech./714). A voucher specimen of the plant has been preserved for future reference in the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Preparation of plant extract

Fresh rhizomes used for extraction were shade dried and powdered. Three hundred gram of finely sieved powder was weighed and subjected to sequential soxhlet extraction at room temperature, using methanol

as the solvent. The extracts were filtered and evaporated to dryness under reduced pressure by a rotary evaporator. After evaporation of the solvent, residues obtained were used for GC-MS analysis.

GC-MS analysis

The Chromatographic analysis was performed on a Shimadzu QP-2010 PLUS gas chromatograph coupled to a mass spectrometer (GC-MS) instrument (Shimadzu Corporation, Japan). The use of a Rtxi-5MS column was employed for the following conditions: Initial temperature was 50°C (held for 3min), increased to 150°C at a rate of 10°C/min (held for 8 min), then to 250°C at a rate of 8°C/min, (held for 2 min), and finally to 280°C at a rate of 10°C/min and held for 5 min. Helium was used as carrier gas and the sample was injected in a splitless mode with a column flow of 1ml/min. Column oven temperature was 50°C and injection temperature was 260°C. The mass spectra were acquired over 40-600 atomic mass unit range with a scan speed of 1250. The individual components were identified by computerized matching of their mass spectra of peaks with those gathered in the NIST 08 and WILEY 8-Mass Spectral library of the GC-MS data software system.

Ligand preparation

By avoiding repeated structures from the crude sample of GC-MS results, 14 total structures were derived which were non-repetitive and without any ambiguities in nature. The chemical structures of the selected compounds were available with PubChem compound database and were downloaded from this database. All computational analyses were carried out on Red Hat 5.2 Linux platform. The compounds were prepared by using LigPrep2.6. The OPLS - 2005 force field was used for optimization, which produces the low-energy conformer of the ligand in maestro format.

Evaluation of Drug likeliness and toxicity prediction

The drug likeliness of the compounds was analyzed using Lipinski's rules. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligands as a drug[24]. Toxicity of the selected molecules was performed using QikProp module from Schrodinger. This includes a principal description and property prediction of the molecule. SMILES notations of the selected compounds were fed in the online molinspiration software version 2011.06 (http://www.molinspiration.com) for prediction of bioactivity score of drug candidates.

Target proteins

The crystal structure of the target proteins of breast cancer (PTEN, STK10, CHEK1, APC, BAX, PALB2, KLRG1, KRAS, BRCT7 and BRCA1) were retrieved from RCSB protein data bank (http://www.pdb.org). Accordingly, the protein structures were retrieved on basis of single monomer, less than 2 Å resolution, *Homosapien* and single domain in the protein complex.

Molecular docking

The possible binding modes between the ligands and the target proteins were studied using Glide 5.9. Glide searches favorable interactions between ligand molecules and the receptor molecule. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The receptor grid generated during protein preparation provides more accurate scoring functions for each ligand

MD Simulation

Desmond Molecular Dynamics system 2.4 with OPLS-2005 all-atom force field was used to perform MD simulations. Desmond is an advanced classical molecular dynamics simulation system, which has an intuitive graphical user interface, integrated into the maestro molecular modeling environment. The systems were equilibrated with the default parameters provided in Desmond. The RMSD and RMSF of both the ligand and protein structures were calculated for the entire simulation trajectory.

RESULTS AND DISCUSSION

ADMET properties are a crucial aspect of the quality of drug candidates. It has become integrated into the drug discovery process and is a tremendous asset in guiding selection and optimization of precious leads. If the properties are weak, the candidate will have a high risk of failure or be less desirable as a product. Out of fourteen selected compounds from the methanol extract of *A.galanga*, 10 molecules were found to satisfy drug-like properties based on Lipinski's rule of five, which is essential for rational drug design. Nine principal descriptors and predicted ADMET properties were calculated for these molecules. Principal descriptors included in the study are HBA (Hydrogen Bond Acceptor), HBD (Hydrogen Bond Donor), MW (Molecular Weight), PSA (Polar surface area), SASA (Solvent accessible surface area), FOSA (Hydrophobic component of SASA), FISA (Hydrophilic component of SASA), PISA (Pi component of SASA) and WPSA (Weakly polar component of SASA) (Table 1).

Table 1
Compliance of principal descriptors of galangal compounds

Principal descriptors	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	Range in 95% of drug
HBA	1	1	2	3	1	1	1	1	7	5	0 - 10
HBD	0	0	1	1	1	0	0	1	3	0	0 - 6
MW	148.20	154.24	160.21	180.20	222.36	152.23	134.17	154.24	374.38	285.29	130 - 725
PSA	26.48	7.23	28.06	56.49	15.29	25.67	38.25	19.13	107.14	77.63	7 - 200
SASA	381.01	377.12	393.29	408.13	469.98	361.20	366.97	396.16	618.58	565.77	300 - 1000
FOSA	161.64	377.12	159.10	212.59	440.44	319.99	89.00	328.47	272.24	126.16	0 - 750
FISA	41.32	0.00	41.51	82.35	14.49	41.20	80.15	31.86	137.83	118.02	7 - 330
PISA	178.04	0.00	192.68	113.19	15.04	0.00	197.81	35.88	208.50	321.57	0 - 450
WPSA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0 - 175

C_1= 3-phenyl-2-butanone, C_2= Eucalyptol, C_3= 1H-Imidazole, 4, 5-dihydro-, C_4= Amfetamine –M, C_5= Cubenol, C_6= Camphor, C_7= Benzene propanal, C_8= alpha terpineol, C_9= Nortrachelogenin, C_{10}= 2-methyl-3-nitophenyl.beta

Hydrogen bond acceptors and donors have a significant role in the descriptor criteria. The number of acceptors and donors influence the passive diffusion of molecules by reducing the partitioning from the aqueous phase into lipid bilayer membrane. Similarly, molecular size also influences passive diffusion. Increasing size of the molecule impedes passive diffusion through the tightly packed side chains of the bilayer membrane[25]. The values of all the ten lead molecules are within the range of acceptable limit. Nortrachelogenin showed excellent descriptor properties. For the prediction of drug transport properties, the surface area is a very useful parameter. Surface area calculations provide results of practically the same quality as the classical calculations, with two to three orders of magnitude faster. PSA describe the Vander Waals surface area of polar nitrogen and oxygen atoms and predicts the passive transport of molecules through membranes. SASA provides a total solvent accessible surface area in square angstroms using a probe with a 1.4Å radius. FOSA and FISA represent the hydrophobic and hydrophilic component of SASA. It is the sum of polar atoms usually saturated carbon, nitrogen, oxygen and attached hydrogen's in a molecule [26]. All these parameters can be correlated very well with human oral bioavailability. PISA describes the π component of SASA contains carbon and attached hydrogen and WPSA is the weakly polar component of SASA contains halogens, phosphorus, and sulfur. Compounds that exceed these values have a higher risk of poor absorption after oral dosing. This will bring to a standstill for pharmaceutical companies to continue investment in their development of drugs. Nortrachelogenin and alpha terpineol have shown to possess good oral bioavailability compared to other compounds.This identification is similar to the findings of Etti *et al.*[6] who employed molecular docking to study the binding affinities of small molecules from the *Artocarpus* species after their drug-like properties were ascertained. The studied ligands were found to be drug-like when compared with 95% of orally available drugs. These properties served as indications of the anti-breast cancer relevance of the studied molecules. Similarly in a comparative docking and ADMET study of some curcumin derivatives established satisfactory results with the majority of cases, the values of principal descriptors were observed within normal range[27]. QikProp tool predicted significant ADMET properties of galangal rhizome through property profiling. Nowadays property profiling has become an important criterion for biological research. Biologists use this data to optimize bioassays, dosing vehicles, and *in vivo* pharmacokinetics. With this understanding, balanced attention towards activity and properties (holistic approach) yields candidates that can become good drugs [25]. For the ten lead molecules, the aqueous solubility (Log S) critical for estimation of absorption and distribution of the drug within the body, are ranged between - 4.163 to -1.622 respectively (Table 2). Solubility is the maximum concentration of a drug that can be reached under the present conditions. It affects the vehicle or formulation of the drug. Many negative effects can occur for low-solubility compounds including poor absorption and bioavailability, development changes and erratic assay results. If bioavailability is low, a higher dosage of the drug is needed to administer[28].

Table 2
Compliance of property predictors of galangal compounds

Property prediction	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**	**C_6**	**C_7**	**C_8**	**C_9**	**C_{10}**	**Range in 95% of drug**
Log S	-2.222	-3.015	-2.404	-1.987	-4.163	-1.990	-1.622	-2.662	-3.624	-3.917	-6.5 / 0.5
Log Kp	-1.462	-1.520	-3.476	-2.254	-1.542	-2.279	-2.012	-1.788	-2.461	-2.082	-8.0 / -1.0
Log Po/w	2.099	2.458	2.191	1.652	4.089	1.938	2.018	2.957	2.559	3.401	-2.0 / 6.5
Log BBB	0.113	0.604	0.104	-0.358	0.328	0.259	-0.268	0.190	-1.268	-0.878	-3.0 / 1.0
Log Khsa	-0.227	0.219	-0.138	-0.385	0.651	-0.181	-0.389	0.118	-0.046	0.251	-1.5 / 1.5
Log HERG	-3.76	-2.613	-4.011	-3.718	-2.978	-2.335	-3.994	-3.054	-5.045	-5.803	Below -5
Metabolism	2	1	1	3	4	1	2	4	7	4	1 / 8
P MDCK	2224.49	5899.29	2214.47	844.66	4190.57	2230.71	889.61	2781.14	228.04	363.93	<25poor,>500 reat
P Caco	4018.15	9906.03	4001.40	1640.39	7219.17	4028.54	1721.00	4940.38	488.47	752.74	<25poor,>500 reat

C_1= 3-phenyl-2-butanone, C_2= Eucalyptol, C_3= 1H-Imidazole, 4, 5-dihydro-, C_4= Amfetamine –M, C_5= Cubenol, C_6= Camphor, C_7= Benzene propanal, C_8= alpha terpineol, C_9= Nortrachelogenin, C_{10}= 2-methyl-3-nitophenyl.beta

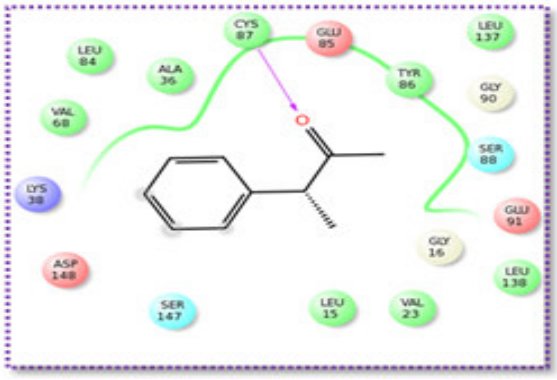
Prediction of dermal penetration is an important factor in drug discovery. Here the computed skin permeability coefficient (Log Kp) presented serves as means for the prediction of transport of galangal phytocompounds through the mammalian epidermis. 3-phenyl-2-butanone and 1H Imidazole represents the maximum and minimum range of values. Log Po/w is used in rational drug design as a measure of molecular hydrophobicity. In the context of pharmacokinetics, the distribution coefficient has a strong influence on ADMET(Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the drug. More specifically, for a drug to be orally absorbed, it normally must clear the transcellular process. For efficient transport, the drug must be hydrophobic enough to partition into the lipid bilayer, but not so hydrophobic, that once it is in the bilayer, it will not partition out again [29]. Brain exposure is assessed in terms of Blood-brain barriers (BBB) or brain/plasma partition. BBB is associated with H-bonds, molecular weight, metabolism and plasma protein binding. Here the predicted BBB for lead compounds is under acceptable range ~ -1.268 to 0.604. Many chemotherapeutic agents used systemically do not cross the blood-brain barrier, or may transiently weaken the BBB and allow extravasations of tumor cells from the circulation into the brain parenchyma. Blecharz *et al* [30] point out that the interaction between the BBB and tumor cells plays a key role in implantation and growth of brain metastases in the central nervous system. Moreover, the BBB, as the tightest endothelial barrier, prevents both early detection and treatment by creating a privileged microenvironment. Therefore, precise targeting the BBB might potentially help to overcome this difficult clinical problem. The predicted value of binding to human serum albumin (Log Khsa) lies in the acceptable range of -0.385 to 0.651. Variation of this calculated parameter may greatly reduce the quantity of the drug in general blood circulation. Our calculations reveal that more than 80% of the lead compounds are compliant to this parameter, indicating that they can circulate freely within the bloodstream and hence have access to the target site. The recommended range for predicted log IC_{50} values for blockage of HERG K^+ channels is below-5. IC_{50} values often provide reasonable predictions for cardiac toxicity of drugs in the early stages of drug discovery [31]. In this work, the predicted value is in the acceptable range below -5. This data can be used in combination with all other data in holistic decision-making process. In recent years, HERG blocking has been one of the leading causes of withdrawal from the market of drugs approved by the FDA. Thus, the cost of clinical development is elevated if a compound has a potential HERG liability. Therefore drug candidates that are HERG blockers require large clinical trials with many patients in order to demonstrate safety. Metabolism is the enzymatic modification of compounds to increase clearance. It is a determinant of oral bioavailability, clearance, and half-life *in vivo*. Metabolism occurs predominantly in the liver, and some may occur in the intestine. MDCK has been actively used in drug discovery for passive diffusion permeability prediction[32]. Our calculated apparent MDCK cell permeability could be a good mimic for non-active transport. Caco-2 also is used to study some transporters because it expresses several transporters of pharmaceutical interest. The most common transporters of interest in drug discovery include Pgp (MDR1), breast cancer resistance protein (BCRP), PepT1, PepT2 and multidrug resistance protein 2 (MRP2). Usually, in wet lab experiments, culture conditions can affect the expression level of transporters. Therefore the functional activity of the transporters should be verified before a cell line is used for screening. Amongst the studied phytocompounds, amfetamine and camphor were less drug-like when compared to the rest. The prediction was done with respect to the oral route of drug administration, which is still the most preferred route for new chemical entities; in spite the advances in drug delivery methods[33]. Previous *in silico* investigation of the pharmacodynamics and pharmacokinetics of xanthone based molecules with target aromatase in breast cancer showed a perfect toxicity profile except for the molecule, Sameathxanthone A which needs further study to improve the ADMET properties[34]. The bioactivity score of the lead compounds was calculated for G - protein coupled receptor (GPCR), ion channel modulator, kinase inhibitor, a nuclear receptor, protease inhibitor and enzyme inhibitor (Table 3). The scores for these compounds can be interpreted as active (bioactivity score > 0), moderately active (bioactivity score: -5.0-0.0) and inactive (bioactivity score < -5.0). Two compounds nortrahelogenin and 1H-Imidazole, 4, 5-dihydro-2, serves to be significant GPCR ligands with a score of 0.25 and 0.11. Bioactivity score for ion channel modulator was in between 0.42 to - 0.50. Kinase inhibitors showed the score in the range of - 0.07 to - 2.11. Molecules with the highest activity score have the highest probability to be active. Nuclear receptor ligand, protease inhibitor and enzyme inhibitors were found to be in the range of -0.02 to 0.28, -0.37 to 0.09 and -0.1 to 0.21 respectively. These properties serve as an indication of excellent pharmacological activities *in vivo* and thus can be used for the development of a new functional drug with less undesirable effects.

Table 3
Bioactivity score of the lead compounds

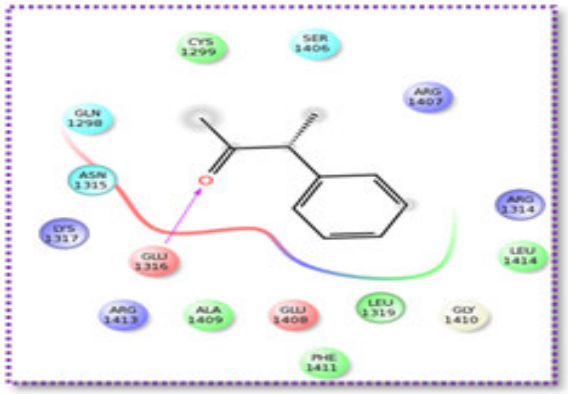
Lead compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
3-phenyl- 2 -butanone	- 1.14	-0.51	-1.40	-0.84	-1.01	- 0.65
Eucalyptol	-0.93	0.01	-1.60	-1.07	-0.90	0.15
1H-Imidazole, 4, 5-dihydro-2 -	0.11	0.42	-0.86	-1.61	-0.57	-0.02
Amfetamine-M	-0.75	-0.42	-0.83	-0.26	-1.28	-0.36
Cubenol	-0.17	0.41	-0.69	0.11	-0.48	0.27
Camphor	-0.79	-0.56	-2.11	-1.21	-0.95	-0.52
Benzene propanal	-0.83	-0.07	-1.23	-0.96	-0.53	-0.30
Alpha terpineol	-0.51	0.15	-1.45	-0.02	-0.78	0.14
Nortrachelogenin	0.25	0.05	-0.07	0.28	0.09	0.21
2-Methyl-3-nitrophenyl.beta	-0.20	-0.22	-0.37	-0.08	-0.37	-0.16

Our observation corroborates with the findings of Khan *et al*. [35] who reported that boswellic acid and its derivatives exhibit good bioactivity score for drug targets including nuclear receptor ligand, protease inhibitor, and enzyme inhibition and thus expected to have excellent pharmacological activity *in vivo*. In another study, the antioxidant compounds present in *Aloe vera* were screened in search of a new lead compound and found that dihydro coumarin ethyl ester showed good drug-likeness score and bioactivity score, in comparison with other compounds and chosen as the best [36]. Veber *et al*.[37] showed that these predicted descriptors correlate well with *in vivo* bioavailability and are critical in developing oral dosage by exploring the possible number of biotransformation of the compounds. The detailed intermolecular interactions between the lead compound and the target protein showed that the ligand, 3-phenyl-2-butanone exhibited gliding interaction with four different breast cancer proteins, PTEN, CHEK1, PALB2 and BRCT7 with the glide score of -4.852, -6.227, -4.143 and -5.670. Another ligand, nortrachelogenin interacts with two breast cancer proteins STK10 and KLRG1 with the glide score of -7.217 and -7.247 and alpha terpineol interact with two breast cancer proteins BAX and BRCA1 with the glide score of -5.425 and -3.707 (Fig. 1).

Figure 1
Ligand interaction diagram with best docked target proteins



3-phenyl-2-butanone - CHEK1



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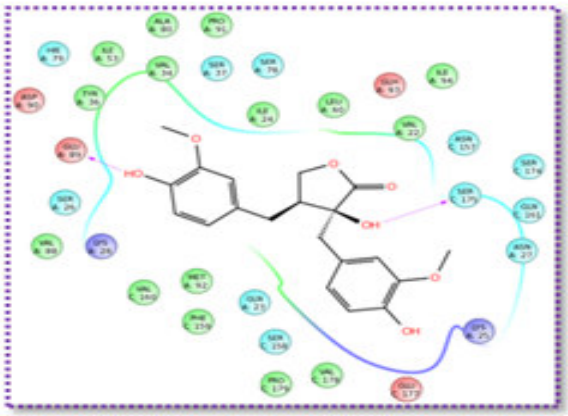
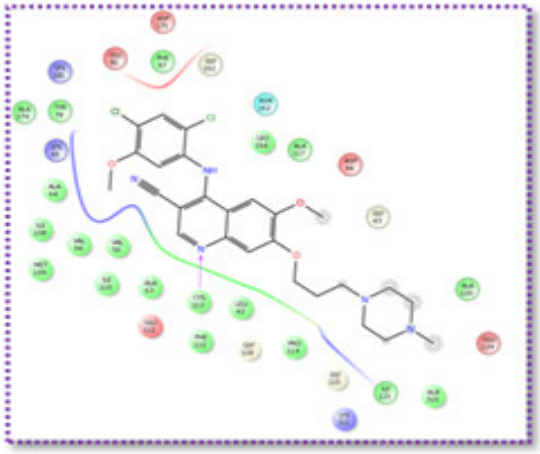
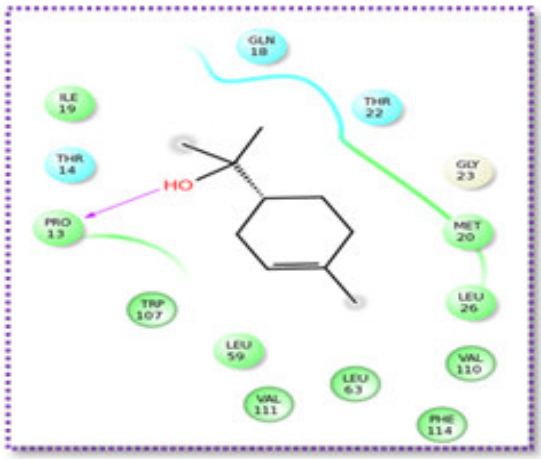


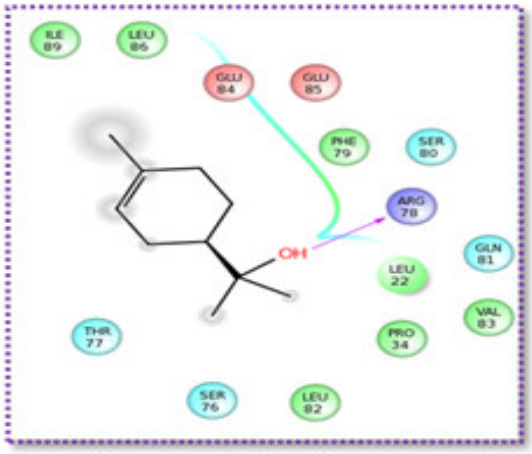
Nortrahelogenin - KLRG1



Nortrahelogenin - STK10



Alpha terpineol – BAX

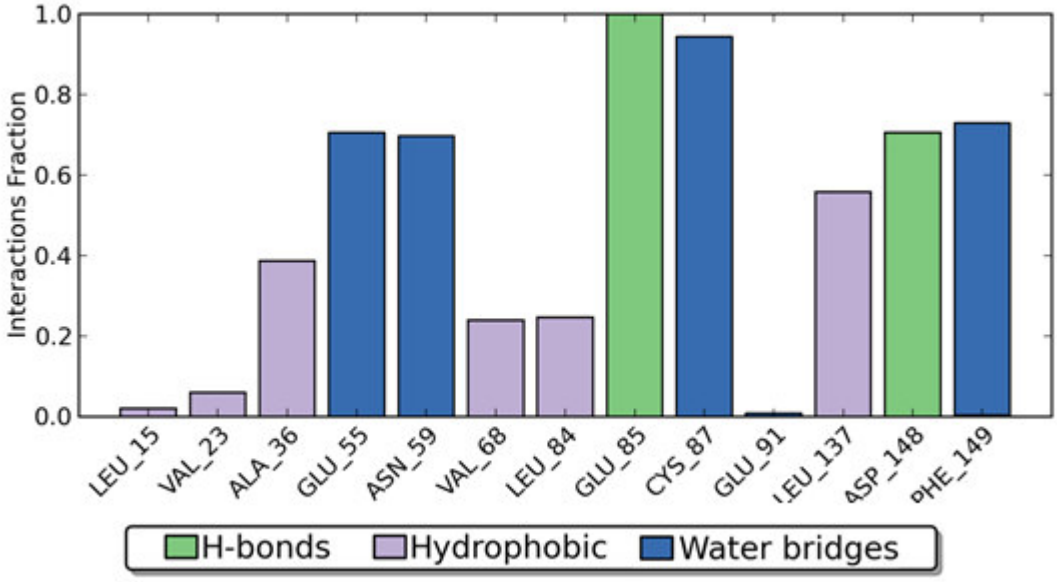


Alpha terpineol - BRCA1

This observation falls in line with the previous report of Murali *et al*. [38] who found that the selected compounds of *Tephrosia purpurea* have notable interaction with various cancer targets. Quercetin exhibited strong affinity with four targets and purpurin with two targets. Similarly the bioactive compounds from the medicinal plant, *Acorus calamus* can act as potential drugs in the early stages of breast cancer detection[39]. Favorable results of docking score indicated the application of curcumin as potential and natural therapeutic agents to treat breast cancer [40]. Suganya *et al.*[41] reported the binding interactions between the human estrogen receptor protein and 19 natural flavonoid compounds. Among the four complexes formed by the same molecule, Checkpoint kinase1 complexed with 3-phenyl-2-butanone exhibited highest glide score and the same complex was subjected for MD simulations. Post-docking validation and MD simulation of protein-ligand complex served as a criterion for assessing interaction profiling. This will lead to the identification of a protein

that has the higher influence to target drug designing and will also pave the way to find the criteria of derivative groups to interact with protein. Usually, a potential energy function helps to model the basic interactions and allows one to gain insight into situations that are impossible to study experimentally. The Chk1 has a central role in coordinating the DNA damage response and therefore is an area of great interest in oncology and the development of cancer therapeutics. Initially, CHEK1 was thought to function as a tumor suppressor due to the regulatory role it serves amongst cells with DNA damage. Later CHEK1 has been shown to be overexpressed in numerous tumors including breast and colon carcinoma [42]. On the basis of its role during DNA damage response, Chk1 has been suggested as an anticancer therapy target[43]. In order to assess the conformational stability of the protein during the simulations, the RMSD from the initial conformation was calculated as a function of time. The RMSD profile of the protein backbone during the simulation period was around 3.0-angstrom units whereas the ligand with reference to itself shows quite stable (0.4 angstroms) up to 500 picoseconds and later found at around 2.8 angstroms. Here all the protein frames are first aligned on the reference frame backbone and the complex was stable within the early 0.5ns production run. Between 0.5 to 1.0 ns, the complex was observed to fluctuate from equilibrium with minor deviation. It was suggested that if the simulation has equilibrated, its fluctuations towards the end of the simulation are around some thermal average structure and the changes of the order of 1-3 Å are perfectly acceptable for small, globular proteins. Similarly, Soureshjani *et al*. [44] analyzed the stability of DNMT1 with the best-docked plant medicine xanthomicrol and compared with those of the respective drug, decitabine and revealed the stabilization of the complexes within 300ns of simulation with better stability of DNMT1. Krishnaveni [45] performed simulation studies of desulphosinigrin as an anticancer drug candidate against cyclin dependent kinase 2 revealed that molecular interactions between the ligand and cyclin-dependent complex were highly stable and the protein was steady throughout the simulation period. The RMSF indicated that the protein residues, Glu, Asn, Cys, Asp, Phe and Leu had greater movement from its native position during the simulation period. The radius of gyration indicated the compactness of the simulated complex. It measures the extendedness of the ligand, which is equivalent to its principal moment of inertia. It was observed that the docked complex from *A.galanga* was compact during the simulation period as it was within the range of 1Å. Good contacts showed that hydrogen bonds maintained 70% of the simulation time in a normal range of 60-85% and also made multiple contacts of the same subtype with the ligand. Hydrophobic contacts maintained more than half of the simulation time (Fig. 2).

Figure 2
Simulation interaction diagram



The current geometric criteria for the protein-ligand H-bond was in a distance of 2.5 Å between the donor and acceptor atoms. A donor angle of ≥120° between the donor-hydrogen-acceptor atoms and an acceptor angle of ≥90° between the hydrogen-acceptor-bonded atoms was observed. Hydrophobic contacts fall into three subtypes: π-cation, π-π, and other non-specific interactions. π -cation to aromatic and charged groups within 4.5 Å, π- π to two aromatic groups stacked face-to-face, other - A non-specific hydrophobic side chain within 3.6 Å of the ligands aromatic carbon atom. For water bridges, the hydrogen-bond geometry of the complex from *A.galanga* was slightly relaxed from the standard H-bond definition. Here the geometric criteria for water-ligand H-bond were in a distance of 2.7 Å. A donor angle of ≥110° between the donor-hydrogen-acceptor atoms and an acceptor angle of ≥80° between the hydrogen-acceptor-bonded atoms was observed. These results are in agreement with the previous reports of Pan *et al*.[46] who described an ab initio molecular dynamic study on hydrogen bonds between water molecules. At many orientation angles and distances, the interaction energies of the two water molecules exceed the energy criterion of the H-bond, but they are

still identified as H-bonded by the conventional distance-angle criteria. In another study Chen *et al*. [47] reported that geometric criteria had a significant effect on hydrogen bonding analysis and the results for glycerol molecules were more sensitive to O-H-O angle and H-O-O angle than that for water molecules.

CONCLUSION

Our *in silico* findings served as a preliminary study providing accurate information for the identification of novel drug candidates. Data highlights the unique potential of phytocompounds in breast cancer therapies. The interaction between the phytocompounds and target proteins proved that nortrachelogenin, alpha terpineol and 3-phenyl-2-butanone effectively inhibited the cancer targets. Being natural, they have minimal or null side effects on human body and thus could be used as promising alternatives.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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