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In Silico Molecular Docking Analysis of Anticancerous Potentials Phytochemicals from *Cymbopogon Citratus*(DC) Stapf against BRCA1 Receptor

	
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ABSTRACT

In recent years, the demands on drug discovery process have been increased dramatically, partly because of the necessity to recognize novel target that are both pertinent to disease and chemically tractable. The emergence of bioinformatics gives room to investigate diseases at the molecular level using computational techniques. The present work was to perform a molecular docking analysis of potential phytochemicals derived from *Cymbopogon citratus* (DC) Stapf into BRCA1 receptor active site to determine the probable binding model against breast cancer. Based on GC-MS data on phytochemicals of *Cymbopogon citratus* (DC) Stapf, 10 compounds were selected as ligands and subjected to molecular docking studies for the inhibition of BRCA1 receptor in the present investigation. The 3D structure of these ligands and BRCA1 receptor were retrieved from online tools Pubchem and PDB respectively. The structure of ligand molecules were drawn using Chemsketch software. Out of 10 bioactive compounds, 4 compounds satisfied the Lipinski's properties. The docking studies were done for these 4 compounds using commercial tool Accelrys Discovery Studio 2.1. Among 4 compounds, linalool showed the highest dock score i.e. 34.067 with low bond length (0.42). The results implied that linalool will act against breast cancer by blocking BRCA1 receptor and it can be developed into a potent drug for breast cancer in future.

INTRODUCTION

Plants constitute major source of drugs for prevention and spread of wide range of pathogenic carriers and also treating various diseases of human beings. Modern people increasingly prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects. Whereas synthetic drugs and antibiotics often cause wide spread toxicity and harmful side effects to the end user other than targeted health condition pathogen carrier. Compounds isolated from plants are safer and have a lot of potential than the chemical drugs⁽¹⁾. In search of novel active compounds from plant origin, and to assess the efficient therapeutic properties with minimum side effects, application of advanced methods like computational techniques play a crucial role in designing and development of drug of interest.

Cancer is a major cause of death and breast cancer is one of the common malignancies and leading causes of cancer death in women around the world. Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too. Wide ranges of carcinogens are responsible for carcinogenicity. Radiation is well documented risk factor for breast cancer and its exposure induces the formation of free radicals. Several improvements in diagnostic protocols enhanced the ability for earlier breast cancer detection with improved therapeutic outcome and survival rate. Breast cancer type 1 susceptibility protein (BRCA1) has been identified as a novel marker for early cancer detection. The multifactorial BRCA1 gene product is involved in DNA repair mechanism, ubiquitination, transcriptional regulation and other functions⁽²⁾.

The most common cause of hereditary breast cancer is an inherited mutation in the *BRCA1* and *BRCA2* genes. In normal cells, these genes help to prevent cancer by making proteins that keep the cells from growing abnormally. If you have inherited a mutated copy of either gene from a parent, you have a high risk of developing breast cancer during your lifetime. Although in some families with *BRCA1* mutations the lifetime risk of breast cancer is as high as 80 %, on average this risk seems to be in the range of 55 to 65 %⁽³⁾. *BRCA1* (breast cancer 1, early onset) is a human tumor suppressor gene that produces a protein called breast cancer type 1 susceptibility protein. Due to the presence of two domains viz. Zinc finger, C3HC4 type (RING finger) and

BRCA1 C Terminus (BRCT) domain, the BRCA1 protein is also known as RING finger protein 53⁽⁴⁾. The *BRCA1* gene is located on 17q21 and has a total length of about 100 kb. This gene consists of 24 exons, and the coding region starts at the middle of exon⁽⁵⁾. The gene product of *BRCA1* is a phosphorylated protein that consists of 1863 amino acids and has a molecular weight of 220kDa⁽⁶⁾.

Docking is a process by which one can predict the significant orientation of one molecule to a second when bound to each other to form a stable complex. Docking is mostly used for finding the binding between the ligand and the receptor⁽⁷⁾. Hence in drug designing docking plays a vital role. Between the two molecules, the binding affinities strength is predicted using the preferred orientation. For docking we require 3D structure of the protein and ligands as the input, for which the bound conformation of the ligand with that of the protein active site is predicted⁽⁸⁾.

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor⁽⁹⁾. Molecular Docking is a great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result, novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions⁽¹⁰⁾. The three dimensional structure of the protein-ligand composite could be served as a considerable source of understanding the way of proteins interact with one another and perform biological functions. Drug-likeness was analyzed as per “Lipinski Rule of 5”⁽¹¹⁾. Hence the present work was carried to perform molecular docking analysis of potential phytochemicals derived from *Cymbopogon citratus* (DC) Stapf into BRCA1 receptor active site to determine the probable binding model against breast cancer.

MATERIALS AND METHODS

Steps in Molecular Docking

Retrieval of Phytochemicals of *Cymbopogon citratus* (DC) Stapf and their 3D structure

Twelve phytochemicals namely Geraniol (Citral A), Neral (Citral B), Nerol, Geraniol, Limonene, β Myrcene, β Caryophyllene, Iso Eugenol, Linalool, α cadinol, α -naphthalene and Elemol were used as ligands in the present investigation. 3D structure of the selected bioactive compounds were retrieved using online tool PUBChem. The three dimensional structures of selected phytochemicals developed by ChemSketch software.

Molecular docking was performed between phytochemicals and receptor using Accelrys Discovery Studio 2.1. Discovery Studio 2.1 was the most advanced computational drug discovery environment available, features significant new science and usability enhancements. It was a single, powerful, easy-to-use, graphical interface for drug design and protein modeling research.

Receptor-Ligand Interactions

The interactions between a receptor and a ligand were fundamental to drug discovery. Discovery Studio provided a set of methods for predicting and analyzing the interactions between protein receptors and ligands. These methods allowed us to carry out structure-based design, or even to examine possible interactions with theoretical structures such as homology models. A common technique central to receptor-ligand interactions was docking.

Discovery Studio provided several docking methods as well as a rich graphical interface to third-party docking tools such as GOLD. Discovery Studio also has included several methods applicable to fragment-based design such as the De Novo protocols. Analysis of hypothetical poses was also possible via a series of scoring functions, hydrogen bonds and bumps, and high level physics-based scoring methods to predict binding energies.

(B) Dock Ligands (Ligandfit)

The Dock Ligands (Ligandfit) protocol in Discovery Studio had three stages:

1. Docking: During Docking, an attempt was made to dock a ligand or series of ligands into a user defined binding site.

2. *In situ* Ligand Minimization: In this stage, the ligands may be energy minimized in the presence of a fixed or partially flexible receptor.

3. Scoring: During scoring, various scoring functions may be applied to ligands. The Dock Ligands (Ligand fit) protocol had allowed us to combine docking, minimization, and scoring in one protocol run. Groups of parameters had allowed us to control the three phases of the protocol: docking, minimization, and scoring.

Retrieval of 3D Structure:

The following steps were used to retrieve the 3D structure.

STEP 1: The Google website was visited and in the search column the keyword RCSB was entered; the search button was clicked.

STEP 2: The RCBS homepage was displayed.

STEP 3: In the search column, enter the receptor name as PBP2A Protein, then the list of receptors were displayed.

STEP 4: From the list, structure of protein was selected and then 3D structure of receptor was saved as 1VQQ.

Selection of ligand:

The following were the steps used in the selection of the Ligand.

STEP 1: The Google website was visited and in the search column the keyword PubChem Compound was entered; the search button was clicked.

STEP 2: PubChem home page was displayed.

STEP 3: In search box enter the compound name.

STEP 4: Select the structure for the compounds in Pubchem.

STEP 5: The selected compounds from Pubchem were drawn by using Chems sketch and saved in .mol format.

STEP 6: Load the structure in discovery studio 2.1.

Docking Process

Before beginning the docking, it was necessary to specify a binding site of the receptor. Ligandfit uses a method based on protein shape searching for cavities. Often the largest cavity was part of the ligand – binding site. The docking process has the following steps:

STEP 1: The water molecules and heta atoms are selected and deleted.

STEP 2: In the structure menu, crystal cell was expanded and removed cell was selected.

STEP 3: In the edit menu, preferences was expanded and clean protein was selected by expanding the protein utilities.

STEP 4: In the tools explorer, the “apply force” was selected.

STEP 5: In the protocols explorer, by expanding the simulation

- a) Minimization was selected.
- b) Dynamics (equilibrium) was selected.

STEP 6: Under the “Binding site” from Tools Explorer, “Define protein molecule as Receptor” was selected.

STEP 7: The “Find sites from Receptor cavities” under the Binding site was selected.

STEP 8: A list of binding sites was opened in the hierarchy view.

STEP 9: The 3-D Structure of Ligand was loaded.

STEP 10: The Receptor-Ligand interaction Protocol was selected.

STEP 11: The other Parameters were set as default.

STEP 12: The RUN Button was clicked for docking process.

STEP 13: The Results were analyzed.

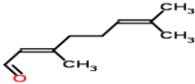
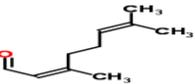
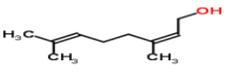
STEP 14: From the results obtained, the least Dock Score Value was chosen.

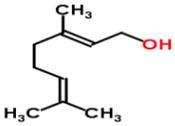
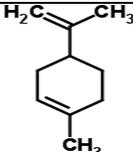
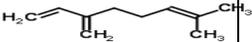
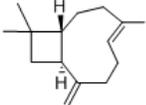
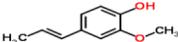
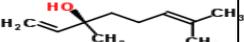
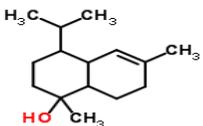
STEP 15: The results were based not only on the Dock Score, but also depends on the Hydrogen Bonding. Here, the molecule with minimum Dock Score was selected first. Then the molecule present in the hierarchy view was also selected. From the tool bar Structure was clicked and Hydrogen Bonds from Monitor was selected. If there is any Hydrogen Bonds, the plus (+) sign was present before Hydrogen Bonds in the hierarchy window which indicates that there was an amino acid interaction between the receptor and the ligand. This was said as Stable Interaction.

RESULTS

Ten phytochemicals of *Cymbopogon citratus* were selected from GC-MS data and the 3D structure of the phytochemicals were retrieved and evaluated for Lipinski's property. Out of 10 compounds 5 compounds namely Linalool, Citral, Limone, Myrcene and Iso-Eugenol satisfied the Lipinski's properties. The Lipinski's properties like molecular weight, log p, number of hydrogen bond donors and acceptors for the phytochemicals of *Cymbopogon citratus* were given in Table 1.

Table 1. Lipinski's properties of the ten compounds of *Cymbopogon citratus* (DC) Staff

Ligand molecule	Molecular weight [g/mol]	Molecular Formula	Xlog p3 value (<=5)	H-bond donor	H-bond acceptor	Structure
Geranial (Citral A)	152.23344	C ₁₀ H ₁₆ O	3	0	1	
Neral (Citral B)	152.23344	C ₁₀ H ₁₆ O	3	0	1	
Nerol	154.24932	C ₁₀ H ₁₈ O	2.9	1	1	
Geraniol	154.24932	C ₁₀ H ₁₈ O	2.9	1	1	

						
Limonene	136.23404	C ₁₀ H ₁₆	3.4	0	0	
β Myrcene	136.23404	C ₁₀ H ₁₆	4.3	0	0	
β Caryophyllene	204.35106	C ₁₅ H ₂₄	4.4	0	0	
Iso Eugenol	250.33338	C ₁₅ H ₂₂ O ₃	4.3	1	3	
Linalool	154.24932	C ₁₀ H ₁₈ O	2.7	1	1	
α cadinol	222.36634	C ₁₅ H ₂₆ O	3.3	1	1	

For each molecule, many orientations and conformations are sampled; based on these configurations, each molecule is scored for complementarity to the receptor and ranked relative to the other members of the database.

Docking analysis

In the present study, docking simulation was performed between BRCA1 with five bioactive compounds from *Cymbopogon citratus* using Accelrys Discovery Studio (2.1) [after Lipinski's rule satisfaction] to find out the binding orientation and binding affinities of the ligand. Out of 5 compounds, 4 compounds showed interaction between ligand and the receptor. The output of all the ligands were given by dock score values was shown in Table 2.

Table 2. Hydrogen bonding interactions between the receptor BRCA1 with ligand molecules

Ligand molecule	Aminoacid	Atoms in aminoacid	Position	Atoms in ligand	H-bond	Dock score
Linalool	LYS GLU THR	HH21 OE2 001	252 341 342	05 H24 H33	5	34.067
Citral	GLU SER THR ARG	HH13 HH22 002 003	344 343 342 351	02 02 H21 H21	4	23.651
Iso Eugenol	LYS GLU SER	01 02 01	252 344 249	H11 H22 H21	3	10.486
Nerol	LYS THR SER	01 02 03	552 553 543	H21 H22 H23	4	21.079

The docking scores were highest for Linalool with docking score 34.067 followed by Geranial (Citral-A) with 23.651. Crucial interaction between the ligands (ash colour) and target protein BRCA1 (red colour) were shown from Figure 1 to Figure 4. The BRCA1 receptor interacting with the ligands were shown in stick model. Moreover, based on the results of molecular docking, the Linalool showed much better binding energy with BRCA1 receptor, and is therefore considered as the most active compound followed by citral.

Summary of the docked pose of 4 anticancer compounds

Figure-I –Linalool

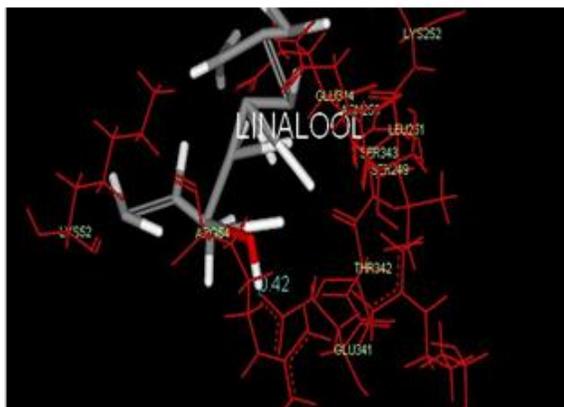


Figure –III-Iso Eugenol

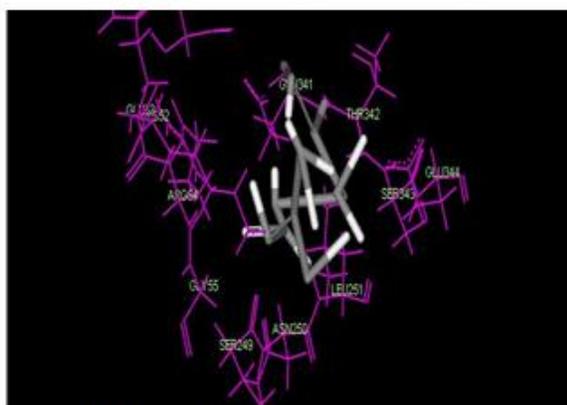
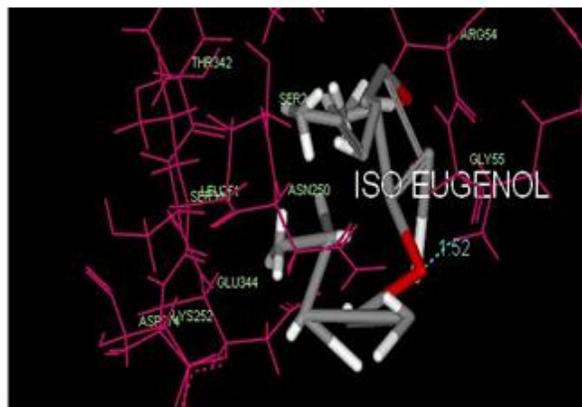


Figure –II-Citral

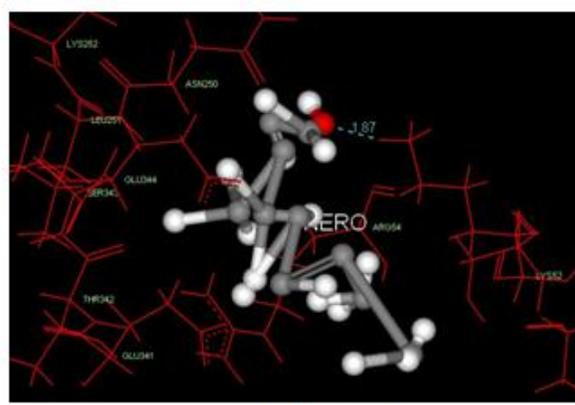


Figure- IV-Nerol

Here top ranked ligands were taken for binding affinity studies. The validation process consisted of two parts: (i) Hydrogen bond details of the top-ranked docked pose and (ii) prediction of Binding energy between the docked ligand and the enzyme using various score calculated using Discovery studio (DJD, LigScore 1, LigScore 2, - PLP 1, -PLP 2, PMF, and JAIN scores were taken for the analysis (Table-3).

Table 3. Summary of docking information of top ranked anticancer compound

Ligand molecule	Lig Score1	Lig Score 2	-PLP1	-PLP2	JAIN	-PMF	Dock score
Linalool	1.22	1.98	12.54	14.78	-1.89	25.36	34.067
Geranial (Citral A)	0.72	3.37	40.62	36.9	1.78	60.76	23.651
Geranial (Citral B)	0.52	2.79	31.6	28.54	-0.47	23.92	21.157
Iso-Eugenol	1.15	3.36	57	57.32	2.99	68.65	10.486
Nerol	0.41	2.49	19.29	20.17	-0.49	17.83	21.709

Here through *in silico* approach it was predicted that the compound Linalool also shown to inhibit BRCA1 as it had good Ligscore and PLP1 when compared to Citral and Limonene. Hydrogen bond formation also makes important contributions to the interactions between ligand and the enzyme. Here a maximum of five hydrogen bonds were formed between the protein and the ligand Linalool followed by four hydrogen bonds were formed between the enzyme and the ligand Citral and Nerol. Thus the concept of protein-Ligand interaction helps in analyzing the binding properties of the protein BRCA1. The study report also concluded that the residues Lys, Glu, Thr, Ser plays an important role in binding mechanism.

DISCUSSION

Molecular Docking continues to hold great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions⁽¹²⁾.

Number of reports citing successful application of CADD in developing specific drugs in different therapeutic areas is expanding rapidly. It is estimated that docking programs currently dock 70 – 80 % of ligands correctly⁽¹³⁾. The most well known factor is the “Lipinski’s rule of

five” which was derived empirically from the analysis of the World Drug Index on the properties that maximize (satisfy) an oral drug candidate’s probability of surviving clinical development. Christopher A. Lipinski formulated Lipinski’s rule of five to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans ⁽¹¹⁾. The rule is important for drug development where a pharmacologically active lead structure is optimized stepwise for increased activity and selectivity, as well as drug like properties as described by Lipinski’s rule. Lipinski’s rule says that in general an orally active drug has not more than one violation of following criteria i.e. has not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient A Log P less than 5 and rotatable bonds of less than 10. Our results were in agreement with Lipinski’s rule of five.

The study suggested that when a drug binds to a target in molecular modelling and molecular design software, high dock score is suitable for better protein-ligand interaction⁽¹⁴⁾. In this view, it is clear from the results (Table 2) that Linalool has a high dock score 34.06 and strong affinity towards the receptor followed by Geranial (Citral-A) with 23.651.

Hydrogen bonding is most likely an essential requirement for many drug-receptor interactions ⁽¹⁵⁾. In this context, it is wise to confirm from Table 4. The interaction between Linalool and receptor conferred a significant amount of stability when compared to Iso Eugenol because Linalool produced five hydrogen bond interactions with the receptors whereas Iso Eugenol produced three hydrogen bond interactions.

The score values include Ligscore1&2 (Protein- Ligand Affinity Energy) ⁽¹⁶⁾, PLP, PLP2 (Steric and H-bonding intermolecular function, Higher PLP scores indicate stronger receptor-ligand binding (larger pKi values) ⁽¹⁷⁾, JAIN (sum of five interactions terms namely Lipophilic interactions, Polar attractive interactions, Polar repulsive interactions, Solvation of the protein and ligand, an entropy term for the ligand) ⁽¹⁸⁾, PMF (developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex, A higher score indicates a stronger receptor-ligand binding affinity) ^(19,20) and Dockscore (Candidate ligand poses are evaluated and prioritized according to the DockScore function) . The determination of

the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein. The two scoring methodologies namely Lig Score and PLP1 were used to estimate the ligand-binding energies. Larger score value indicates better ligand-binding affinity. The study results were in agreement with these score values.

CONCLUSION

The docking analysis of potential phytochemicals derived from *Cymbopogon citratus* (DC) Stapf into BRCA1 receptor active site was done to determine the probable binding site against breast cancer using commercial tool Accelrys Discovery Studio 2.1. Newly synthesized compounds have shown promising dock value. To strengthen the current investigation, further evidences both *in vitro* and *in vivo* are needed so as to use this approach effectively for cancer treatment.

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