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GC-MS Analysis of Ethanolic Extract of *Borerria hispida L.* and *in-silico* Analysis against Breast Cancer Targets

P. Thenmozhi[1]* and T.V. Poonguzhali[2]

[1]Department of Plant Biology and Plant Biotechnology, JBAS College for Women, Chennai-18, India
[2]Department of Plant Biology and Plant Biotechnology, Queen Mary's College, Chennai-4, India

KEYWORDS

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Docking,
ER Alpha
and
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A B S T R A C T

Cancer is a neoplasmic deadly disease that involves unregulated cell division and tissue invasiveness. Existing lines of cancer treatment include surgery, radiation, and chemotherapy. These modern lines of treatment produce serious side effects. Recent studies established that herbs and herbal medicine are free from serious side effects. The aim of the present study is to ethanolic plant extract subjected GC-MS analysis and Docking. In the present investigation, The GC-MS analysis revealed the presence of thirty one compounds. These compounds were subjected Lipinski rule of five and pre admet analysis for identifying potent breast cancer leads. Breast cancer targets were downloaded from RCSB PDB for docking studies. Lead compounds were selected for Docking studies based on preadmet insilico analysis. Using Discovery studio 4.0 the selected compounds docked with Breast cancer targets. The ethanolic extract of *Borerria hispida* contains 7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(2-methyloxiranyl)- shows best docking score against Breast cancer target.

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 (Greer *et al*., 2003). 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 (Schapira *et al*., 2002).

Traditional herbal medicine is a rich source for modern, molecular target specific drug discovery (Thakur *et al*., 2005). Plants have proven to be the most useful in curing diseases and provide an important source of pharma and medicine. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human (Paterson, 1998). The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development (Miki *et al*., 1994). Medicinal plants are the foundation of many important drugs of the modern world. Many of these local medicinal plants are used as spices and food items. Plants based drug is the major area of research. According to WHO calculations 80% of the world's population presently uses medicinal herbs drug for their primary health care (Ruffner *et al*., 1997). Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide (Venkatachalam *et al*., 2003).

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 (Rarey *et al*., 1995). 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 (Gschwend *et al*., 1996).

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 (Irwin *et al*., 2006). 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 (Lipinski *et al*., 2001). 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" (Irwin *et al*., 2005). Current chemotherapeutic drugs are not useful in all cases and have severe side effects on human health. So there is increased demand in identification of new plant based drugs. With the view to identify a herbal drug source for the development of anti cancer drug from *Borerria hispida L.* is selected and studied.

Materials and Methods

Plant collection

Borerria is a procumbent, branched, hairy herb, 10 to 14 centimeters long. Branches are greenish or purplish, ascending, stout and 4-angled. Leaves are ovate, spatulate, or elliptic, 1 to 3.5 centimeters long, 0.8 to 1.7 centimeters wide, pointed or rounded at the tip. Flowers are 4 to 6, occurring in whorls in the axils of leaves. Calyx-teeth are linear-lanceolate. Corolla is white, 5 to 10 millimeters in length. Fresh plants of the *Borerria* were collected from Thiruvannamalai and identified using flowers and authenticated by Retired Professor, Anatomist Dr Jayaraman, Thambaram.

Extraction of plant material

Plant materials were shade dried and coarsely powdered. Measured amount of air-dried powdered plant materials was taken in an aspirator bottle and was soaked in alcohol for 2 days at room temperature. On 3rd day the extract was distilled off and residue subjected to further analysis. alcohol was added subsequently in the order of increasing polarity and extracts were obtained after distilling off the solvents. Then the extracts obtained were filtered and evaporated using a vacuum rotary evaporator at 40°C.

GC-MS analysis

For the Identification of bioactive components in extract with greater antioxidant activity, the extract was subjected to GC-MS analysis. GC-MS analysis was carried out on a GC-MS - 5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature 2400C; ion-source temperature 2000C. The oven temperature was programmed from 700C (isothermal for 2 min), with an increase of 100C/min, to 3000C/min, ending with a 9 min isothermal at 3000C. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 2000C and interface temperature being 2400C (Fig.1)

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the

component of the test materials were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998).

ProteinData Bank (PDB)

PDB abbreviated as protein data bank was originally developed by Brookhaven National Laboratory in 1971. From 2003 it was maintained by worldwide Protein Databank (wwPDB). The wwPDB members are RCSB PDB (USA), PDBE (UK), PDBJ (Japan) and the BMRB (USA). It contains archive of information about the 3D structure of biomacromolecules and their complexes determined by X-ray crystallography, NMR spectroscopy and cryo-electron microscopy. The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function.

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 ER Alpha Protein, then the list of receptors were displayed.

STEP 4: Three dimensional structure of the ER alpha receptor was retrieved from database using its id Protein preparation The ligand and crystallographic water molecules were removed from the protein (Fig 1)

Pubchem

Pubchem database provides information about the biological activities of small ligand molecule. It comprises three linked database such pubchem compound, pubchem substance and pubchem bioassay. The PubChem Compounds Database contains validated chemical depiction information provided to describe substances in PubChem Substance. Structures stored within PubChem Compounds are pre-clustered and cross-referenced by identity and similarity groups. Three dimensional structure of the desmosterol phytocompound was retrieved through pubchem text search and the structure was downloaded in .sdf format. Ligand preparation The three dimensional structure of phytocompounds saved in .sdf format were converted to mol farmet (Table 1)

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.

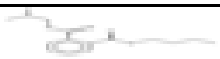
STEP 4: Select the structure for the compounds in Pubchem.

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

STEP 6: Load the structure for Docking using Discovery studio 4.0.

Twelve phytocompounds namely Geranial (Citral A), Neral (Citral B), Nerol, Geraniol, Limonene, β Myrcene, β Caryophyllene, Iso Eugenol, Linalool, α cadinol, α-napthallene 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 phytocompounds developed by ChemSketch software.

Table.1 shows GCMS ethanolic extraction of Phytochemicas

S. No.	Retention Time	Peak Area %	Compound Name	PubChem ID/ Nist -Cas R.No	Structure
1	16.654	2.58	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl	10129	
2	17.099	0.92	1,4-Eicosadiene	5365774	
3	17.099	0.92	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans	1124-27-0	
4	17.099	0.92	7-Thiebicyclo [4.1.0] heptane, 2-methyl-	96-08-2	
5	17.872	1.11	Dibutyl phthelate	3026	
6	17.872	1.11	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	17851-53-0	
7	19.366	1.47	Phytol	5280435	
8	24.996	4.50	Squalene	638072	
9	25.588	2.67	Nonacosane	12409	
10	25.588	2.67	Eicosane	18936	
11	25.588	2.67	Heneicosane, 11-decyl	143269	
12	26.682	1.47	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	51794-16-2	
13	26.682	1.47	squalene	141-79-7	
14	26.682	1.47	2-Methyl-3-[3-methyl-but-2-enyl]-2-[4-methyl-pent-3-enyl]-oxetane	550119	
15	27.292	3.53	Vitamin E	14985	
16	27.292	3.53	Beta-Tocopherol, O-methyl-	6857447	

S. No.	Retention Time	Peak Area %	Compound Name	PubChem ID/ Nist -Cas R.No	Structure
17	29.148	14.50	gamma s-Sitosterol	83-47-6	
18	29.149	14.50	beta-Sitosterol	83-46-5	
19	29.320	2.29	2-Ethylacridine	610161	
20	29.320	2.29	Benzene, 2-[[tert-butyldimethylsilyl]oxy]-1-isopropyl-4-methyl	62790-85-6	
21	29.438	2.87	Benzo[h]quinoline, 2,4-dimethyl-	610182	
22	29.438	2.87	Cyclotrisiloxane, hexamethyl-	73145	
23	29.676	14.38	Beta-Amyrin	73170	
24	29.676	14.38	Alpha-amyrin	610159	
25	29.676	14.38	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0.0(1,6)]dodec-10(11)-en	160867438	
26	29.869	2.14	Cyclo-2,5- hexa-diene-1,4-dione, 2-methyl -3- (4-morpholinyl)-	10129	
27	30.144	3.33	Azobenzene, 3,10-dihydro-9,9,10-trimethyl	330371	
28	30.144	3.33	1,2,5-Oxadiazol-3-amine,4-(4-methoxyphenoxy)-	1554159	
29	30.256	37.87	Olean-12-ene, 3-Methoxy)-	612798	
30	30.256	37.87	Urs-12-ene	612603	
31	30.876	4.36	9,19-cycloergost-24(28)-en-3-ol,14-dimethyl-,(3.beta.,4.alpha.,5.alpha)-	101690	

Fig.1 Mass spectram of ethanolic extract of Borreris hispida

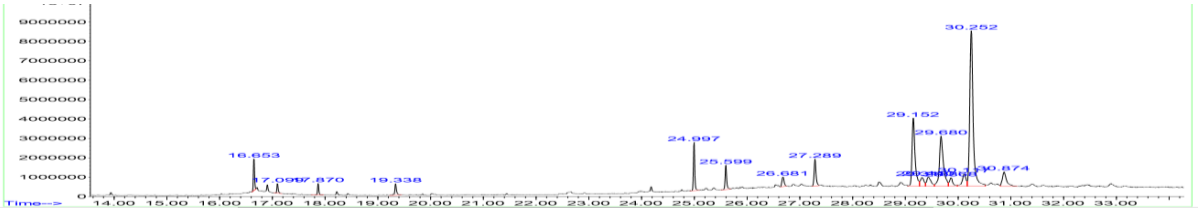


Fig.2. 7 Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyl) shows interaction with ER Alpha

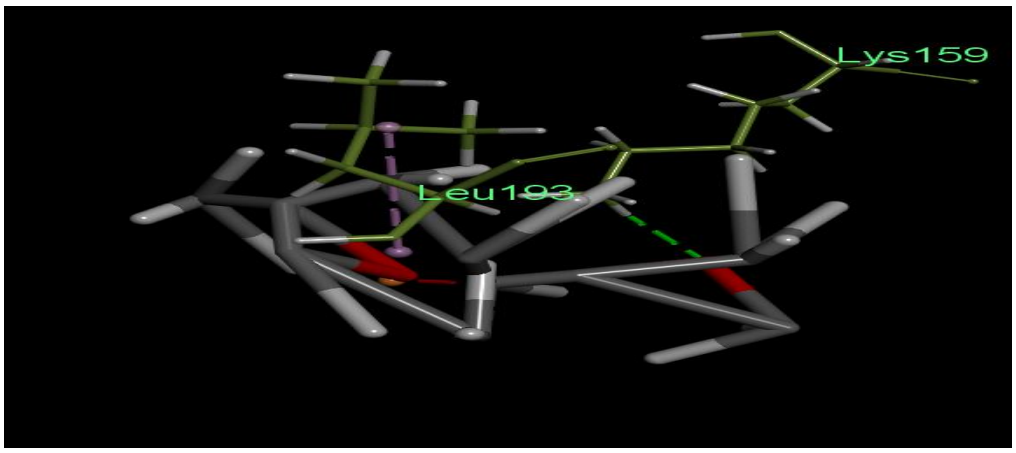
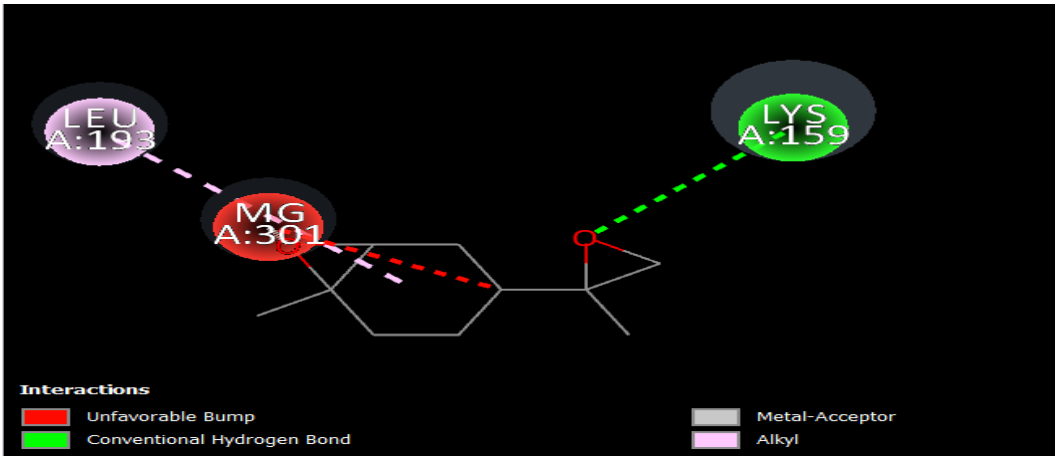


Fig.3.7 Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyl) shows interaction with ER Alpha



Molecular docking was performed between phytocompounds and receptor using Accelrys Discovery Studio 4.0. Discovery Studio 4.0 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. The results were based not only on the Dock Score, but also depends on the Hydrogen Bonding. Here, the molecule with maximum Dock Score was selected. Then the hierarchy window indicates that there was an amino acid interaction between the receptor and the ligand (Fig.2 and Fig.3)

Results and Discussion

Nine phytocompounds of *Borerria hispida* were selected from GC-MS data and the 3D structure of the phytocompounds were retrieved and evaluated for Lipinski's property. Out of 32Seven lead compounds were selected for Docking studies based on preadmet insilico analysis. Using Discovery studio 4.0 the following compounds Bicyclo[3.1.1]heptane,2,6,6-trimethyl,

Cyclohexane,1-methyl-4-(1-methylethenyl)-trans, 7-Thiabicyclo [4.1.0]heptane,2-methyl-, Cyclo-2,5- hexa-diene-1,4-dione, 2-methyl -5- (4-morpholinyl)-, Squalene, 2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane, supraene, Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl, Beta-Amyrin, Alpha-amyrin, Olean-12-ene, 3- Methoxy)-, Urs-12-ene and 9,19-cycloergost-24(28)-en-3-ol,14-dimethyl-,(3.beta.,4.alpha.,5.alpha)- docked with Breast cancer targets.

Docking analysis

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 (LigScore 1, LigScore 2, -PLP 1, -PLP 2, PMF, and JAIN scores were taken for the analysis). The protein-Ligand interaction helps in analyzing the binding properties of the protein ER Alpha. The study report also concluded that the residues Lys 159 and Leu193plays an important role in binding mechanism.

Conclusion

The docking analysis of potential phytocompounds derived from *Borreria* into receptor active site was done to determine the probable binding site against breast cancer using ER Alpha using tool Discovery Studio 4.0. New compound 7-**Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyl)have shown promising dock value from the ethanolic** extract of *Borreria*. 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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