In Silico Molecular Docking and ADME/T Analysis of Selected Bioactive Compounds from *Leucas Zeylanica* Against Breast Cancer

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Abstract

Breast cancer is the most common invasive cancer and the second leading cause of cancer death in women. It develops from the breast tissue and the signs may include a lump in the breast, dimpling of the skin, a change in breast shape, fluid coming from the nipple etc. From the literature review, it had been found that estrogen receptor alpha (ER-α) is one of the main responsible cause for breast cancer. The study aimed to screen out the effective bioactive compounds from Leucas zeylanica. Thirty seven compounds were identified by gas chromatography–mass spectrometry and among them, fourteen bioactive compounds were selected for in silico analysis against ER-α using Schrödinger Suite-Maestro v10.1.Among all the compounds 3-azabicyclo [3.2.2] nonane showed the best docking score towards ER-α. So, 3-azabicyclo [3.2.2] nonane may be the best compound for selective inhibitors of ER-α. Further in vivo investigations are needed to identify potent lead compounds.

Key Words: Breast cancer; *in silico*, *Leucas zeylanica*, estrogen receptor alpha; Schrödinger Suite-Maestro v10.1.

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1. Introduction

Breast cancer, with a frequency of 10.4% percent is the utmost prevalent type of cancer where normal breast cells have the receptors to bind estrogen and progesterone circulating in the blood.[1] These blood circulating hormones bind to the receptors and produce a growth response in the form of a signal cascade leading to cell proliferation and growth. Their role in cancerous cells cannot be undermined as estrogen function with oncogenes and tumor suppressor genes causing the cell to grow out of control.[2] Breast cells that are estrogen and progesterone receptor positive (i.e., ER+ and PR+) are more likely to respond hormonal therapy and have a better prognosis than cancers that are hormone receptor negative. Recently it has been studied that the compounds from natural sources are of great importance for their ant carcinogenic properties because of their less toxicity and vast abundance[3] and fortunately 35% of all of the cancer cases can be addressed by the variation of dietary habits as well as phytochemicals for BCs.[4,5] In addition, the phytochemicals have high selective biological actions and they are easy to extract.

By reviewing literature it was found that, different plants of subfamily Lamioideae and family Lamiaceae have anticancer activity specially *Leucas aspera*[6] but less attention has been paid on the plant *Leucas zeylanica* regarding bio-active compounds isolation and anticancer activity specially activity against breast cancer.

Leucas zeylanica, commonly known as Ceylonslit wort, is a herbaceous aromatic plant which is small, annual, tufted, terrestrial in nature and is of the family Lamiaceae. The herb isbitter in taste and the indigenous tribes use *L. zeylanica* for fever, jaundice, and for scorpion and snake bites.[7]

Through the process of gas chromatography-mass spectrophotometry (GC-MS) several compounds had been isolated for molecular docking. Molecular docking methodologies are of terrific importance in the making plans and layout of new drugs. These strategies goal to expect the experimental binding mode and affinity of a small molecule within the binding site of the receptor target of

interest. A successful docking methodology must be able to correctly predict the native ligand pose the receptor binding site (i.e., to find the experimental ligand geometry within a certain tolerance limit) and the associated physical-chemical molecular interactions.[8]

The principal aim of this study was to find out theeffective bioactive compounds from the GC-MS compound list of *L. zeylanica*, which could be the probable inhibitor of estrogen receptor alpha (ER-α).

2. Materials and Methods

2.1. Protein Preparation

Three-dimensional crystal configuration of estrogen receptor alpha (PDB id: 3ERT) was downloaded in pdb (Protein Data Bank) file format from the protein data bank. The structure was further refined with the help of protein preparation wizard of Schrödinger Suite-Maestro v10.1. Furthermore, the bond orders and charges along with the addition of hydrogen to the heavy metals, selenomethionines conversion to the methionine were done and at last water were deleted by the use of force field OPLS_2005 and minimization was carried out by setting maximum heavy atom root mean-square-deviation to 0.30 Å.[9]

2.2. Ligand Preparation

The gas chromatography helps to separate mixture of components into individual and mass spectrometry identifies molecules based on their mass. So, GC-MS is a chemical analysis technique combining two instruments to provide for powerful separation and identification. From GC-MS thirty-seven (37) compounds had been identified (Figure-1) and among which only fourteen (14) bioactive compounds were enlisted and subjected to play their role as ligands. Their lists as well as structure and PubChem Id number is given in (Table-1).

Figure-1: Total ionic chromatogram (TIC) for the methanolic extract of the aerial parts of *L. zeylanica* via gas chromatography–mass spectrometry.

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Sample Information

Analyzed by : Admin

Analyzed : 6/18/2019 4:09:52 PM

Sample Type : Unknown

Sample Name : LZ

Sample ID : LZ

Injection Volume : 1.00

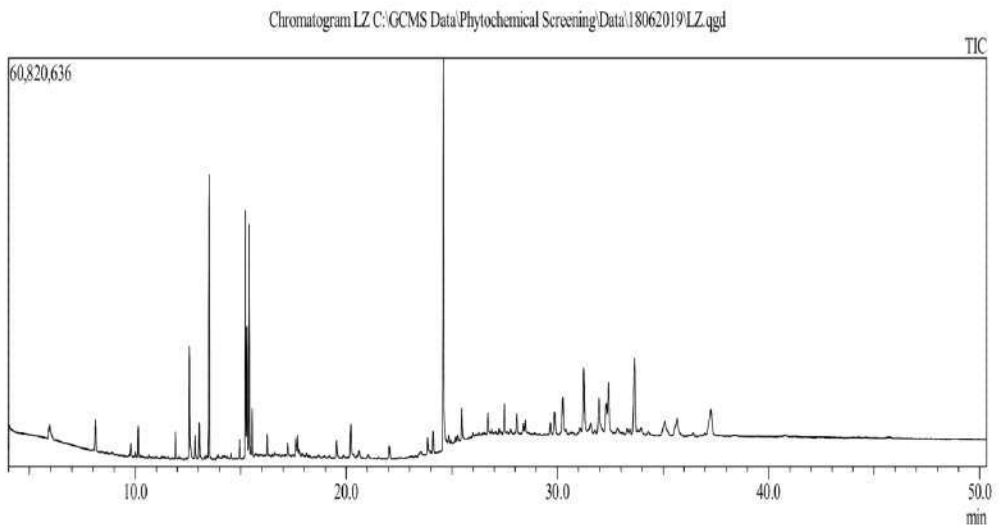
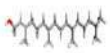
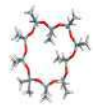
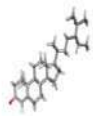


Table-1: Compounds list and structure (2D) along with their PubChem Id number.

Sl. No.	Compound Name	Compound structure(2D)	PubChem Id(CID)
01.	3,7,11,15-tetramethyl-2-hexadecen-1-ol		5366244
02.	3-azabicyclo[3.2.2]nonane		5384531
03.	Methyl 6-octadecenoate		11109696
04.	Methyl (E)-octadec-6-enoate		5284421
05.	11-oxa-dispiro[4.0.4.1]undecan-1-ol		558562
06.	13-docosenamide		5365371

07.	Eicosamethylcyclodecasiloxane		519601
08.	Tetradecamethylcycloheptasiloxane		7874
09.	Octadecamethylcyclononasiloxane		11172
10.	Methyl hexadecanoate		8181
11.	Methyl stearate		8201
12.	Phytyl acetate		6428538
13.	Stigmast-4-en-3-one		5484202
14.	11-bromoundecanoic acid methyl ester		223913

Compounds were retrieved from PubChem databases (Access date: 11.8.2019). The 2D structures for these were built by using Ligprep2.5 in Schrödinger Suite 2015 with an OPLS_2005 force field. Their ionization states were generated at pH7.0±2.0 using Epik2.2 in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

2.3. Receptor Grid Generation

Receptor grids were analysed for prepared proteins such that various ligand poses bind within the predicted active site through docking. In Glide, grids were created keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS_2005 force field. A cubic box of specific dimensions placed round the centroid of the active site residues was generated for the receptor. The bounding box was set to 15 Å × 15 Å × 15 Å for docking experiments.

2.4. Glide Standard Precision (SP) Ligand Docking

With the help of Schrödinger Suite-Maestro v10.1 the ligand docking was carried out which were furthermore applied to non-cis/trans amide bonds and the van der Waals scaling factor and partial charge and cut-off were selected.[10,11] Final scoring was presented on energy-minimized poses and displayed as Glide score. The best-docked pose with lowest Glide score value was recorded for each ligand.

2.5. ADME (absorption, distribution, metabolism, and excretion) and Toxicity Analysis

The pharmacokinetic characteristics of all identified phyto compounds were evaluated and screened for drug candidacy using Lipinski's rule of five (RO5). According to Lipinski's RO5, a compound may exhibit optimal drug-like behavior if the selected parameters are within the specified limit and do not violate more than one of the following five criteria: molecular weight <500 g/mol; ≤5 hydrogen bond donors; ≤10 hydrogen bond acceptors; lipophilicity <5; and molar refractivity between 40 and 130. The web-based tool Swiss ADME, which is considered to be a convenient drug discovery tool, was used to analyze the drug likeness criteria of the detected biological compounds. Compounds that pass Lipinski's RO5 can be considered suitable candidates for

the development of new drug entities.[12] For the evaluation of Pharmacokinetics (ADME) analysis the absorption, distribution, metabolism, and excretion of the compounds 3-azabicyclo [3.2.2] nonane, 11-bromoundecanoic acid methyl ester, methyl stearate, 11-oxa-dispiro [4.0.4,1] undecan-1-ol along with their toxicity were evaluated.

3. Results

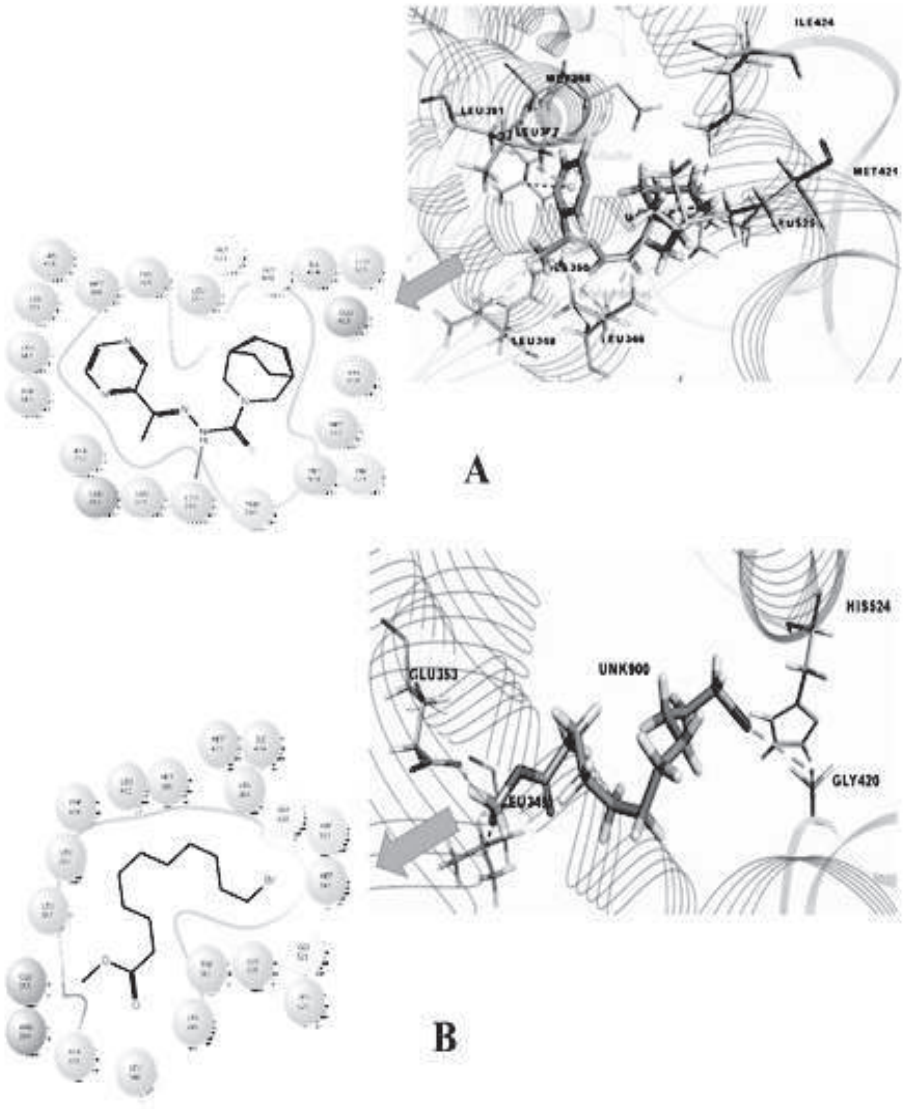
3.1. *In silico* Molecular Docking Analysis

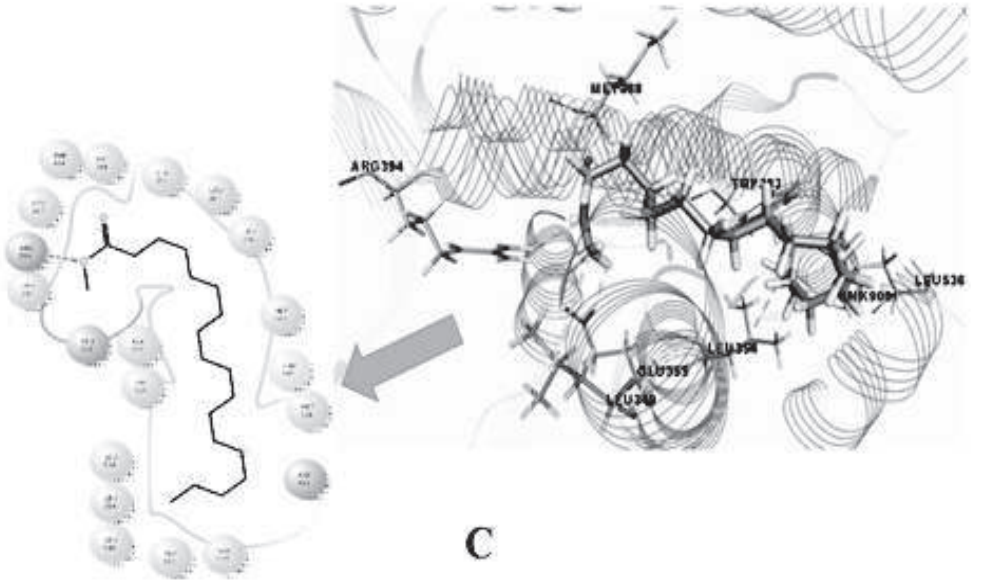
The use of computational techniques for virtual screening has a significant impact on the drug discovery process. With the utilization of docking and scoring of each of the compounds from a set of data, the prediction of binding modes and binding affinities of the ligand to the receptor can be optimized by means of docking to an X-ray crystallographic structure.[13] Grid created docking study was used to investigate the binding modes of molecules with the amino acids adjacent in the active pocket of the protein.[14] In order to study the interaction of the compounds 3-azabicyclo [3.2.2] nonane, 11-bromoundecanoic acid methyl ester, methyl stearate, 11-oxa-dispiro [4.0.4,1] undecan-1-ol with ER-α we performed Glide docking analysis by Schrödinger Suite-Maestro v10.1., where among of these compounds 3-azabicyclo [3.2.2] nonane showed highest docking score shown in Table-2. The negative and low value of free energy of binding demonstrates a strong favourable bond between3ERT (ER-α receptor) and3-azabicyclo [3.2.2] nonane in most favourable conformations. As lower the binding energy values, stable is the complex. Here the result reflects least binding with Methyl stearate and as binding energy is a positive value as that means the binding is energy consuming and it occurs only if the required energy was available. The results of docking analysis are shown in Table-2 and Figure-2.

Table-2: Docking score of 3-azabicyclo [3.2.2] nonane, 11-bromoundecanoic acid methyl ester, methyl stearate, 11-oxa-dispiro [4.0.4,1] undecan-1-ol with ER-α.

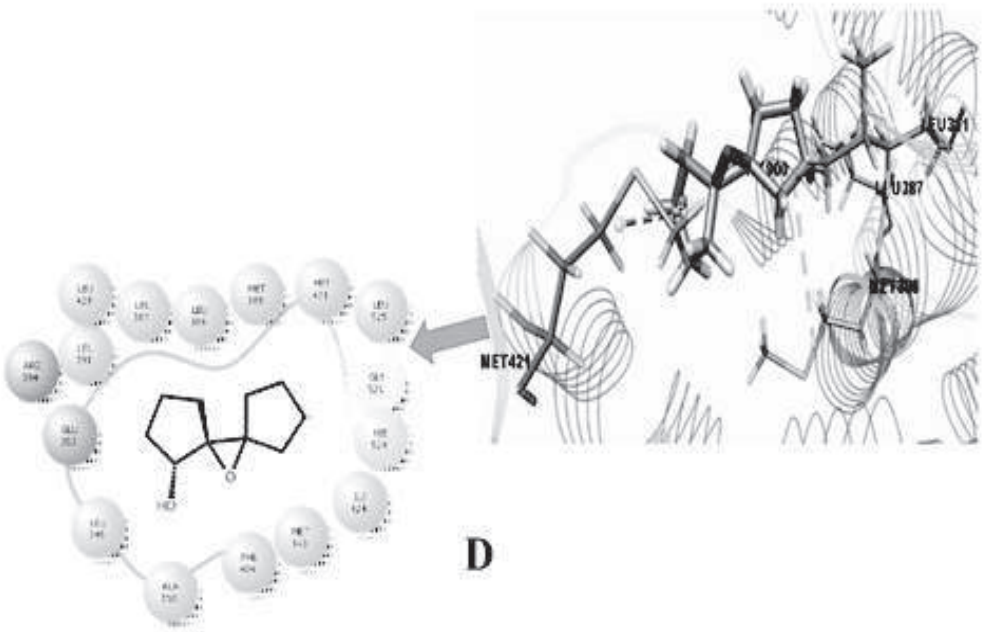
Sl. No.	Name of the Compound	Docking Score
1.	3-azabicyclo [3.2.2] nonane	-7.989
2.	11-bromoundecanoic acid methyl ester	-2.342
3.	Methyl stearate	1.389
4.	11-oxa-dispiro [4.0.4.1] undecan-1-ol	-7.034

Figure-2: 2D and 3D figures showing 3-azabicyclo [3.2.2] nonane binding with ER-α (A), 11-bromoundecanoic acid methyl ester binding with ER-α (B), Methyl stearate binding with ER-α (C), 11-oxa-dispiro [4.0.4,1] undecan-1-ol binding with ER-α (D)





C



D

3.2. ADME and Toxicity Analysis

The ADME properties of the 3-azabicyclo [3.2.2] nonane, 11-bromoundecanoic acid methyl ester, methyl stearate, 11-oxa-dispiro [4.0.4,1]undecan-1-ol are shown in Table-3. The selected properties are well-known to influence cell permeation, bioavailability and metabolism. Here, predicted properties of these four compounds are found in the range to satisfy the Lipinski's rule of five to be recognized as drug like potential.

Table-3: Toxicity and ADME analysis of the compound isolated from *L.zeylenica* by Swiss ADME analysis.

Compound name	Molecular weight[a] (g/mol)	H-donor[b]	H-acceptor[c]	LogP value[d]	Molar refractivity[e]
3-azabicyclo [3.2.2] nonane	125.21	1	1	1.99	43.06
11-bromoundecanoic acid methyl ester	279.21	0	2	4.26	68.95
Methyl stearate	298.50	0	2	6.72	94.73
11-oxa-dispiro [4.0.4,1] undecan-1-ol	168.23	2	2	2.42	46.16

[a]Molecular weight accepted range < 500, [b]Hydrogen bond donor acceptable range ≤ 5,[c]Hydrogen bond acceptor acceptable range ≤ 10, [d]High lipophilicity (expressed as LogP, Acceptable range < 5), [e]Molar refractivity should be between 40 -130.

4. Discussion

Medicinal plants have been used as ideal sources of therapeutic agents for thousands of years. Through computational studies, researchers can predict the activities of several phytocompounds that might be effective for several diseases. From the statistics, it has been seen that breast cancer is the second major cause of death in case of women and the main cause of this cancer is the over expression of estrogen receptor alpha. Hence, it is necessary to find out the compounds which have the higher binding affinity with estrogen receptor alpha to stop their over expression and ultimately for the prevention of breast cancer.[15] By the process of molecular docking, the researchers get an idea to virtually screen out of structures to predict the strongest binding scores computer aided drug designing (CADD) which helps the researcher through decreasing the time and money for drug designing projects.[16]

Molecular docking is very helpful in studying the interactions of ligand molecules with the target protein before their *in vitro* tests. Docking is performed through computer programs like Maestro.[11,12,17] In the current study, we docked several identified phytochemicals present in *L. zeylanica* methanolic extract with the estrogen receptor alpha (PDB ID: 3ERT) with the help of Schrödinger Suite-Maestro v10.1. The methyl stearate had shown lesser binding affinity with estrogen receptor alpha whereas 3-azabicyclo [3.2.2] nonane showed the highest binging affinity in comparison with 11-bromoundecanoic acid methyl ester and 11-oxa-dispiro [4.0.4.1] undecan-1-ol.

There are specific ranges to study the ADME/T analysis i.e., molecular weight accepted range should be less than 500 dalton, there should be less than 5 hydrogen donors, the hydrogen bond donor acceptable range should be less than or equal to 5,high lipophilicity is acceptable (expressed as LogP, acceptable range should be less than 5) and molar refractivity should be between 40 -130.[18] From the results, it can be suggested that the ADME/T profile of the four bioactive compounds were in range according to Lipiniski's rule and as the

targeted natural compounds are also within the reported criteria, indicating that these compounds may be evaluated as potential drug molecules.

5. Conclusions

We used a methodology for computer-aided drug design to detect potent estrogen receptor alpha (ER-α) inhibitors following the *L. zeylanica* phytochemical analyses. Our research showed that several of the identified phyto compounds from *L. zeylanica* followed Lipinski's RO5 and were therefore assessed as promising therapeutic molecules. Among the bioactive compounds, four compounds docked with the targeted receptor ER-α and among them 3-azabicyclo [3.2.2] nonane showed highest docking score and may be the best compound for selective inhibition of estrogen receptor alpha. Due to a lack of trust in scoring algorithms to provide correct binding energies, molecular docking is severely constrained. This is due to the fact that some intermolecular interaction parameters, such as solvation effect and entropy change, cannot be predicted properly. Following additional investigations in biological laboratories, these compounds may possibly be developed into effective therapeutic candidates against breast cancer.

6. Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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