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ANTICANCER POTENTIAL OF ISOLATED PHYTOCHEMICALS FROM *OCIMUM SANCTUM* AGAINST BREAST CANCER: *IN SILICO* MOLECULAR DOCKING APPROACH

Mohammed Farhad[1,2], Md. Imam Hossain Bhuiyan[1], Sheikh Montasir Uddin[1], Md. Hosne Mobarak Chowdhury[1], Md. Nazmul Huda[1], Mohammad Forhad Khan[1], Md. Shahajahan Alam[1], Arkajyoti Paul[2,3], Mohammad Shah Hafez Kabir[1,2]*, Md. Mominur Rahman[1,2]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh.

[2]GUSTO A Research Group, Chittagong-4000, Bangladesh.

[3]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong-4000, Bangladesh.

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***Corresponding Author**
Mohammad Shah Hafez Kabir
GUSTO A Research Group,
Chittagong-4000,
Bangladesh.

ABSTRACT

Breast cancer is an increasing public health problem. One of the main causes of breast cancer is estrogen receptor alpha. Over expression of estrogen receptor is seen in number of cases of breast cancer. The aim of this study was to screen out the effective bioactive compounds from *Ocimum sanctum* namely Carvacrol, Palmitic Acid, Stearic Acid, Vicenin, which may be potential inhibitors of estrogen receptor alpha (ER-α) for searching a drug against the breast cancer. A wide range of docking score found during molecular docking by Schrodinger. Carvacrol, Palmitic Acid, Stearic Acid, Vicenin showed the docking score -6.456, 2.232, 1.985, -2.941 respectively. Among all the compounds Carvacrol showed best docking score towards estrogen receptor alpha. So, Carvacrol is the best compounds for selective inhibitors of estrogen receptor alpha, as it possessed best value in Molecular docking. Further *in vitro* and *in vivo* investigation need to identify estrogen receptor alpha inhibitory activity of isolated compounds from *Ocimum sanctum*.

KEYWORDS: *Ocimum sanctum*, estrogen receptor alpha, Breast cancer, Carvacrol.

INTRODUCTION

Breast cancer remains a major public health problem. The incidence is rising in most countries and is projected to rise further over the next 20 years despite current efforts to prevent the disease.[1-3] The increased incidence is not surprising since there has been, in most countries, an increase in numbers of women with major breast cancer risk factors, including lower age of menarche, late age of first pregnancy, fewer pregnancies, shorter or no periods of breastfeeding, and a later menopause. Other risk factors which add to the burden of breast cancer are the increase in obesity, alcohol consumption, inactivity, and hormone replacement therapy (HRT).[4] One of the main causes of breast cancer is estrogen receptor alpha. Over expression of estrogen receptor is seen in number of cases of breast cancer. The classical estrogen pathway is the direct binding of estrogen-responsive elements by using ligand-activated ER to alter gene expression. Estrogen may also act as a co-activator of other transcription elements to show on oncogenes in breast most cancers in the non-classical pathway.[5-7] Moreover, estrogen can stimulate fast, extranuclear (nongenomic) signaling occasions, along with the activation of the Src/Ras/Erk signaling pathway.

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]

Ocimum sanctum L. (also known as *Ocimum tenuiflorum*, Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. Tulsi, the Queen of herbs, the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The sacred basil, Tulsi, is renowned[9] for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. It is mentioned by Charaka in the Charaka Samhita; an Ayurvedic text. Tulsi is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of 'elixir of life' and believed to promote longevity. Tulsi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning

and malaria. Traditionally, *O. sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects.[10] Tulsi is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil. Tulsi is an important symbol in many Hindu religious traditions, which link the plant with Goddess figure. The name 'Tulsi in Sanskrit means 'the incomparable one'. The presence of a Tulsi plant symbolizes the religious bend of a Hindu family.

The aim of this study was to screen out the effective bioactive compounds from *Ocimum sanctum*, which may be potential inhibitors of estrogen receptor alpha (ER-α) in future and may act as a drug which may be effective in preventing the breast cancer.

MATERIALS AND METHODS

Protein Preparation

Three-dimensional crystal Structure of estrogen receptor alpha (PDB id: 3ERT) was downloaded in pdb format from the protein data bank.[11] After that, the structure was prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v10.1. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionines, and all waters were deleted. Using force field OPLS_2005, minimization was carried out setting maximum heavy atom RMSD (root-mean-square-deviation) to 0.30 Å.

Ligand Preparation

Compounds were retrieved from PubChem databases, i.e. Carvacrol, Palmitic acid, Stearic acid and Vicenin. The 3D 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.

Receptor grid generation

Receptor grids were calculated for prepared proteins such that various ligand poses bind within the predicted active site during docking. In Glide, grids were generated 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 centered around the centroid of

the active site residues (Reference ligand active site) was generated for the receptor. The bounding box was set to 14 Å × 14 Å × 14 Å for docking experiments.

Glide Standard Precision (SP) ligand docking

SP flexible ligand docking was carried out in Glide of Schrödinger-Maestro v 10.1[12,13] within which penalties were applied to non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff were selected to be 0.80 and 0.15, respectively for ligand atoms. Final scoring was performed on energy-minimized poses and displayed as Glide score. The best-docked pose with lowest Glide score value was recorded for each ligand.

RESULTS

In silico Molecular Docking analysis

Advances in computational techniques have enabled virtual screening to have a positive impact on the discovery process. Virtual screening utilizes docking and scoring of each compound from a dataset and the technique used is based on predicting the binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure.[14] Grid based docking study was used to analyze the binding modes of molecules with the amino acids present in the active pocket of the protein.[15] In order to study the interaction of the compounds, like Carvacrol, Palmitic Acid, Stearic Acid, Vicenin with estrogen receptor alpha (ER-α). We performed Glide docking analysis by Schrodinger suite v10.1, where among of these compounds Carvacrol shows best docking score shown in Table 1. The negative and low value of free energy of binding demonstrates a strong favorable bond between 3ERT and Carvacrol in most favourable conformations. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1.

Table: 1 Docking results of Carvacrol, Palmitic Acid, Stearic Acid, Vicenin with estrogen receptor alpha (PDB: 3ERT).

Compound Name	Docking Score	Glide emodel	Glide energy
Carvacrol	-6.456	-28.526	-20.008
Palmitic Acid	2.232	-17.822	-21.365
Stearic Acid	1.985	-11.158	-15.68
Vicenin	-2.941	-44.993	-39.759

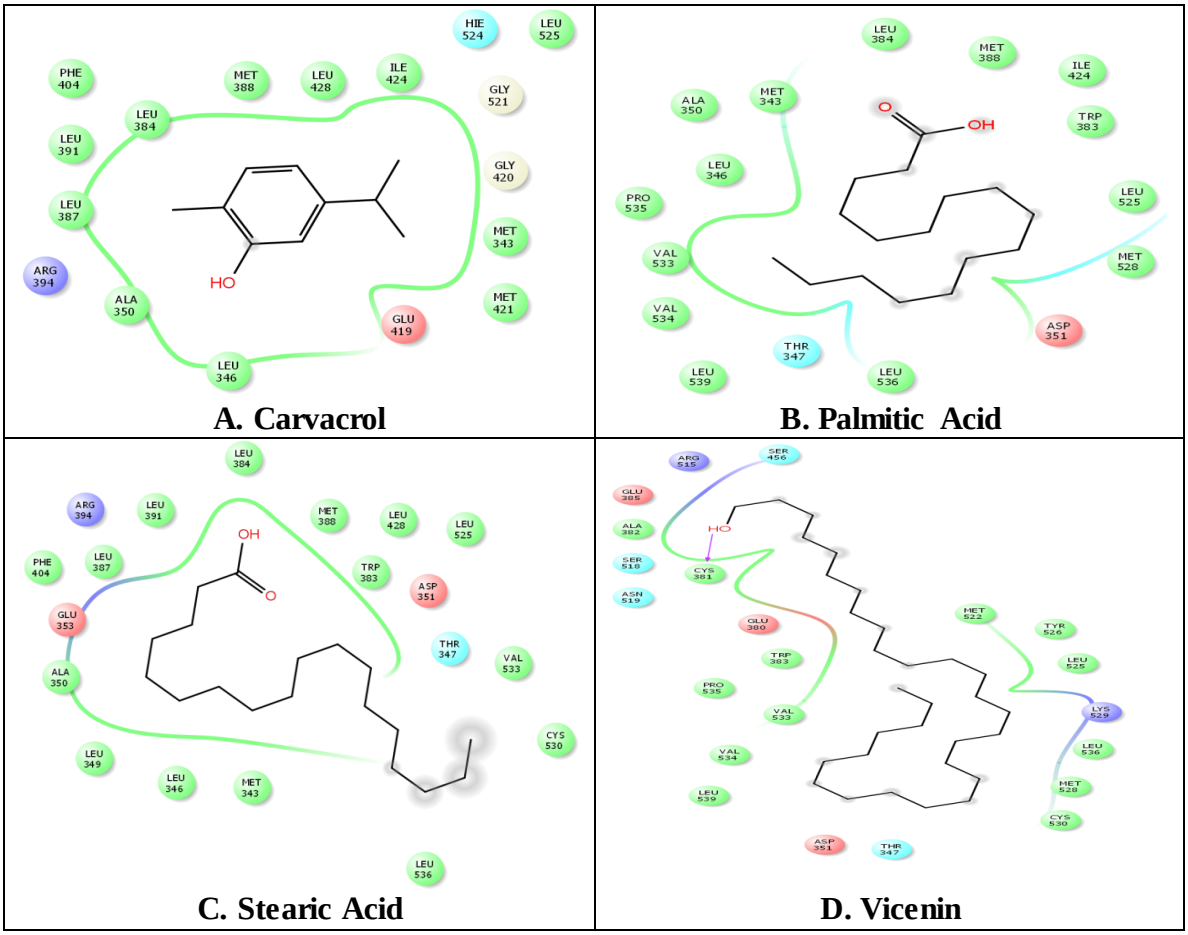

Figure: 1 Docking results of A. Carvacrol, B. Palmitic Acid, C. Stearic Acid, D. Vicenin with estrogen receptor alpha (PDB: 3ERT).

DISCUSSION

Breast cancer is known as a death sentence and second major cause of death in world. Ratio of breast cancer in is one in nine in case of women.[16] Main cause of breast cancer is over expression of estrogen receptor alpha.[17] Therefore ER-α is used as a target for prevention of breast cancer. Tamoxifen is an antagonist of ER-α and commercially available as a drug to control the breast cancer.[18] It binds with Arg394 and blocks the function of estrogen receptor and inhibits the function of ER-α.[19]

Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme Human estrogen receptor fit together and dock to each other well, like pieces of a three-dimensional jigsaw puzzle. The molecules binding to a receptor, inhibit its function, and thus act as drug. In recent research, computer aided drug designing (CADD) helps the researcher to decrease the time and money for drug designing

projects.[20] Molecular docking is very helpful in studying the interactions of ligand molecules with the target protein before its *in vitro* synthesis. Docking is performed through computer programs like Maestro.

To screen out the effective bioactive compounds from *Ocimum sanctum* namely Carvacrol, Palmitic Acid, Stearic Acid, Vicenin, which may be potential inhibitors of estrogen receptor alpha (ER-α) for searching a drug against the breast cancer. We performed Glide docking analysis by Schrodinger suite v10.1. A wide range of docking score found during molecular docking. Carvacrol, Palmitic Acid, Stearic Acid, Vicenin showed the docking score -6.456, 2.232, 1.985, -2.941 respectively. Among of these compounds Carvacrol shows highest docking score shown in Table 1. The negative and low value of free energy of binding demonstrates a strong favorable bond between 3ERT and Carvacrol in most favourable conformations.

CONCLUSION

Among all the compounds Carvacrol showed best docking score towards estrogen receptor alpha. So, Carvacrol is the best compounds for selective inhibitors of estrogen receptor alpha, as it possessed best value in Molecular docking. Further *in vitro* and *in vivo* investigation need to identify estrogen receptor alpha inhibitory activity of isolated compounds from *Ocimum sanctum*.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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