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

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ABSTRACT

Breast cancer is an increasing public health problem. One of the main causes of breast cancer is estrogen receptor alpha. Overexpression of estrogen receptor is seen in a number of cases of breast cancer. The aim of this study was to screen out the effective bioactive compounds from *Grevillea robusta* namely 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid, 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. 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid showed the docking score -6.49, -6.187, -7.368 and -6.103 respectively. Among all the compounds Grasshopper Ketone showed best docking score towards estrogen receptor alpha. So, Grasshopper Ketone is the best compounds for selective inhibitors of estrogen receptor alpha, as it possessed the best value in Molecular docking. Further *in vitro* and *in vivo* investigation need to identify estrogen receptor alpha inhibitory activity of isolated compounds from *Grevillea robusta*.

KEYWORDS: *Grevillea robusta*, estrogen receptor alpha, Breast cancer, 4-hydroxyacetophenone.

INTRODUCTION

Breast cancer is the most common cancer among Western women, with approximately 1.67 million cases diagnosed annually worldwide.[1] 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 the 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 and inactivity.[2] One of the main causes of breast cancer is estrogen receptor alpha. Overexpression of estrogen receptor is seen in a number of cases of breast cancer.

Breast cancer usually starts off in the inner lining of milk ducts or the lobules that supply them with milk. A malignant tumor can spread to other parts of the body. A breast cancer that started off in the lobules is known as lobular carcinoma, while one that developed from the ducts is called ductal carcinoma. The vast majority of breast cancer cases occur in females. Breast cancer is the most common invasive cancer in females worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. 18.2% of all cancer deaths worldwide, including both males and females, are from breast cancer.[3]

In silico biology refers to computational models of biology, is an expression used to mean performed on a computer or via computer simulation. It is used in systems biology. Due to the vast amounts of data that is now generated by molecular and cell experimental biologists, computational biology is increasingly necessary to manage it. Molecular docking is an *in silico* approach. 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.[4]

The chemotherapeutic agents used for its treatment are derived from the plant origin, particularly fruits, leaves, flowers, lichens and fungi. “Herb” is a botanical term means plants

producing fruits, seeds, with non-woody stems. These plants and herbs have played very important role in maintaining the human health. Today public has more interest in herbal remedies than synthetic medicines because herbals contains natural active compound that can support the human health.

Grevillea robusta, commonly known as the southern silky oak or silky oak, or Australian silver oak, is the largest species in the genus Grevillea of the family Proteaceae. It is not closely related to the true oaks, Quercus. It is a native of eastern coastal Australia, in riverine, subtropical and dry rainforest environments receiving more than 1,000 mm per year of average rainfall. *Grevillea robusta*. A. Cunn.exr.Br also known as savUku[5] in Tamil belongs to Proteaceae family. It is an Australian silk oak, commonly planted as ornamental in many warm temperate and semi-tropical climates. It is distributed in the tropical highlands of India. It has been planted extensively as shade for tea and coffee.[6, 7] It is frequently used as a wind break although opinions differ as to its wind firmness and branch shedding tendencies.[8] Silk-oak is an important honey tree in India where it is also regarded as a good fuel wood producer 8. The tree produces an attractively figured easily worked wood, which was once a leading face veneer in world trade, where it was marketed as "lacewood". The wood contains an allergen that causes dermatitis for many people 9. With a view to locating additional flavonoids, the flowers of *G. robusta* have been investigated and the results were presented here under.

The aim of this study was to screen out the effective bioactive compounds from *Grevillea robusta*, 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.[9] 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. 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, and P-coumaric acid. 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 on 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[10, 11] 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 data set by means of docking to an X-ray crystallographic structure.[12] 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.[13] In order to study the interaction of the compounds, like 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid with estrogen receptor alpha (ER-α). We performed Glide docking analysis by Schrodinger suite v10.1, where among of these compounds Grasshopper Ketone shows best docking score shown in Table 1. The negative and low value of free energy of binding demonstrate a strongly favorable bond between 3ERT and

Grasshopper Ketone in most favorable conformations. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1.

Table 1: Docking results of 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid with estrogen receptor alpha (PDB: 3ERT).

Compound Name	Docking Score	Glide emodel	Glide energy
4-Hydroxyacetophenone	-6.49	-36.017	-26.071
Bisnorstriatol	-6.187	-43.677	-28.082
Grasshopper Ketone	-7.368	-44.712	-32.703
P-Coumaric Acid	-6.103	-39.052	-28.84

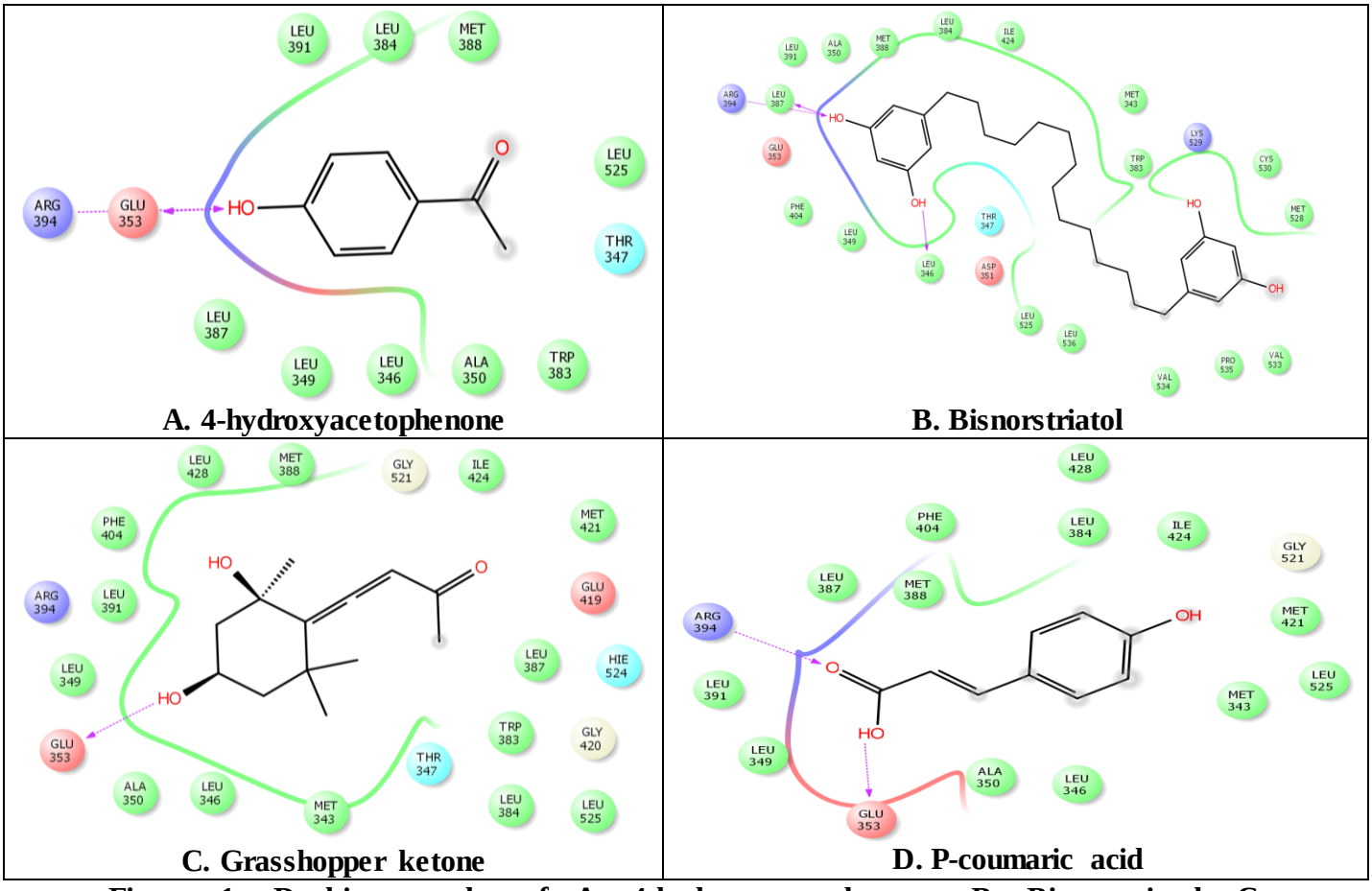

Figure 1: Docking results of A. 4-hydroxyacetophenone, B. Bisnorstriatol, C. Grasshopper ketone, D. P-coumaric acid with estrogen receptor alpha (PDB: 3ERT).

DISCUSSION

Ancient Egyptians were the first to mention castor oil as a medicine and since then this oil, also known as Palma Christus, has been used as a folk medicine.[14,15] Castor oil packs had been highly popularized by Edgar Cayce, "The Sleeping Prophet", who recommended, in

particular, to eradicate tumors near the breast surface. The seed oil from the castor bean *Ricinus communis* is very rich in Δ-12-hydroxy-9-octadecenoic acid (ricinoleic acid, about 90%) and contains as minor components phenolic compounds, such as p-coumaric acid, ferulic acid, o-coumaric acids, syringic, cinnamic, chlorogenic, neochlorogenic and gallic acids. And according to estimates from the World Health Organization, 80% of the world population, especially those living in Asia, Latin America and Africa, still relies on herbal medicine. So, from the natural source, potential breast cancer treatment can be found.

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 a drug. In recent research, computer aided drug designing (CADD) helps the researcher to decrease 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 it's *in vitro* synthesis. Docking is performed through computer programs like Maestro.

To screen out the effective bioactive compounds from *Grevillea robusta* namely 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid, 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. 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid showed the docking score -6.49, -6.187, -7.368 and -6.103 respectively. Among of these compounds Grasshopper Ketone shows highest docking score shown in Table 1. The negative and low value of free energy of binding demonstrate a strongly favorable bond between 3ERT and 4-hydroxyacetophenone in most favorable conformations.

CONCLUSION

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

COMPETING INTERESTS

The authors declare that they have no competing interests.

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