# Anti-Cancer Activity of *Orthosiphon stamineus* Against the Target Protein ERα in Breast Cancer by *Insilico* Docking Studies

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**ABSTRACT**

Breast cancer is one of the most common cancer and is second only to lung cancer as a cause of cancer death in women. Plant-derived compounds have played an important role in the development of clinically important anticancer agents. *Orthosiphon stamineus* is one of the traditionally used medicinal plants belonging to Lamiaceae family, exhibiting a wide range of pharmacological properties. In this work, the plant compounds were identified from the literature survey and its activity were validated against breast cancer target protein ER α through *in*silico docking method. Totally seven phenolic compounds were identified and Molecular docking studies were performed against the target protein. The compound structures were taken from PubChem database and the 3-dimensional structures of the proteins were obtained from PDB databank. Molecular docking study was performed and the result revealed that from the seven compounds, two compounds were found to have the best binding affinity greater than -7.5kcal/mol against ER α. Thus, this study concluded that these two compounds may act as good inhibitors and serve as an important lead compound against breast cancer target protein ER α.

**Keywords**: Breast Cancer, *Orthosiphon stamineus*, Docking.

## INTRODUCTION

In spite of good advancements for diagnosis and treatment, Cancer has still become a big threat to human beings globally. Breast cancer is one of the most common cancers and is second only to lung cancer as a cause of cancer death in women. Statistics regarding breast cancer reveal that 1 in 8 women will develop this cancer during their lifetime[1]. The incidence of breast cancer increases with advancing age. Both personal and family histories influence a woman's risk of developing breast cancer. Factors that may explain the increase in incidence include increased use of screening mammography and use of postmenopausal hormone-replacement therapy (HRT). Germ-line mutations in either BRCA1 or BRCA2 are associated with an increased risk for breast cancer[2].

The main treatment for breast cancer is surgical management like radical mastectomy, breast conservation surgery etc. This is followed by radiation and chemotherapy to prevent relapse[3]. The hormone receptors clinically useful in discussions of breast cancer include the estrogen receptor (ER) and the progesterone receptor (PR). Hormone receptors are not strong prognostic markers, but are used clinically to predict response to hormone therapy. The abnormal expression of Estrogen Receptor α-positive is found to be associated with about 70% of the primary breast cancer patients[4,5].

The Estrogen Receptor alpha (ERα) plays an important role in signaling network and regulating cell proliferation and differentiation[6,7]. Hence, the inhibition of Estrogen Receptor has become a major approach for preventing and treating breast cancer[8]. Various drugs used in breast cancer, target this receptor and current drugs used in the management are tamoxifen, toromifene, anastrazole, exemestane, letrazole etc. These drugs improve survival in cancer patients with positive estrogen receptor. But they are associated with various adverse effects such as uterine cancer, blood clots, stroke etc[9]. These adverse effects make the need for the necessity of new improved drugs by exploring an alternative approach using *In*silico docking method to find out new drug lead compound from herbal plants. Docking studies are currently

gaining popular in identifying lead compounds for treatment of various diseases like malaria, tuberculosis, cancers etc[10]. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health. Medicinal plants play a fundamental role in the world health, since they are sources of several pharmacological active compounds[11]. Plants are reported to have anticancer properties and they are frequently considered to be less toxic and have fewer side effects than synthetic ones. The anticancer drugs obtained from plant sources and are now used commonly, for various cancers include vinca alkaloids, etoposides, taxanes etc[12].

*Orthosiphon stamineus*, belongs to the Lamiaceae family and is traditionally used in Southeastern Asia as an herbal tea. Its medicinal indications include hepatitis, epilepsy, menstrual disorder, renal calculi, gallstone, nephritis, arthritis, antipyretic and diabetes[13,14,15,16]. Moreover, it has been scientifically proven that *O. stamineus* exhibits a wide range of pharmacological properties such as anti-oxidant, anti-tumor, diuretic, anti-diabetic, anti-hypertensive, anti-inflammation, anti-bacterial, and hepatoprotective activities[17,18,19]. Literature survey reveals that the seven phenolic compounds namely Guanosine, Tetra o methyl scutellarein, Di alpha tocopherol, Squalene, Stigmasterol, N,n-diacetylurea, Glycerol were responsible for these above activities[20,21].

The aim of the present study is to assess the strength of interactions between these seven compounds and breast cancer target ER alpha, by calculating the minimum binding energy (kcal/mol) between them using *in*silico docking method.

**MATERIALS AND METHODS**
**Preparation of macromolecule**
The Protein target ER α which is mostly responsible for causing Breast cancer is identitied and the 3D structure of this protein with PDB id 2IOG, were retrieved from the protein data bank (PDB) (http://www.rcsb.org/pdb/)[22]. Minimized structures were saved in PDBQT file format that contains a protein structure with hydrogen in all polar residues.

**Preparation of Ligands**
The seven compounds identified from the plant *Orthosiphon stamineus* were retrieved by using the Pubchem Compound data base and saved in the sdf format[22]. Then its energy form were minimized and converted to pdbqt format by Open Babel in PyRx 0.8

**Lipinski rule of five**
Using the Lipinski rule of 5 server (http://www.scfbio-iitd.res.in/ software/drug design/lipinski.jsp) Molecular properties and drug likeness of the compounds was examined on the basis of "Lipinski's Rule of Five" (Lipinski et al., 2001)[23]. The Lipinski's rule, formulated by Christopher A Lipinski in 1997 is a rule of thumb to evaluate drug likeness which states that an orally active drug has no more than one violation of following criteria i,e., molecular weight below 500 Daltons, log P less than 5, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, Molar refractivity should be between 40-130.

**Identification of Binding site**
The identification of ligand binding sites on target protein surface was carried out with metaPocket (http://metapocket.eml.org)[24]. It is a consensus method, in which the binding sites can be predicted by eight methods (LIGSITEcs, PASS, Q-SiteFinder, SURFNET, Fpocket, GHECOM, ConCavity and POCASA), combined together to improve the prediction success rate.

**Docking studies using Autodock vina PyRx**
PyRx is virtual screening open source software used for Computational Drug Discovery. It is useful in screening of various compounds against potential drug targets with already established software such as AutoDock Vina. AutoDock Vina does not need to calculate the grid maps and to assign atom charges. The predicted binding affinity of bound structures is given in kcal/mol.[25]

**RESULTS AND DISCUSSION**
**Structure of the target protein and ligands**
The three dimensional structure of the cancer target was retrieved from the Protein Data Bank with PDB ID: 2IOG, determined by X-Ray crystallography were visualized in Pymol and shown in Fig.1. The seven compounds identified from the plant *Orthosiphon stamineus* were retrieved by using the Pubchem Compound data base were shown in Table 2.

**Lipinski rule of five of the ligand molecule**
Molecular properties and drug likeness of the seven compounds was examined on the basis of "Lipinski's Rule of Five" were shown in the Table 1.
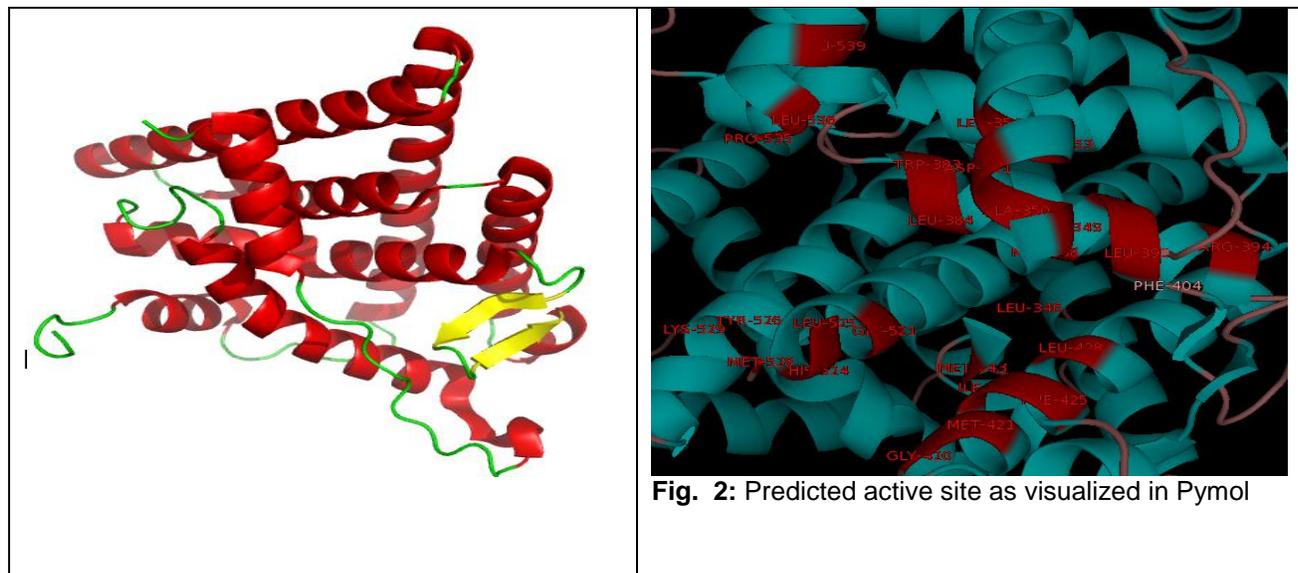
**Fig. 2:** Predicted active site as visualized in Pymol

**Table 1: Shows the Lipinski rule of five for all the seven compounds**

| Compound | molecular weight below 500 Daltons | log P less than 5 | not more than 5 hydrogen bond donors | not more than 10 hydrogen bond acceptors | Molar refractivity should be between 40-130. |
|---|---|---|---|---|---|
| Squalene | 410 | 4.62 | 0 | 0 | 56 |
| Guanosine cyclic monophosphate | 345.21 | -2.87 | 5 | 8 | 100 |
| Stigmasterol | 412.70 | 3.87 | 1 | 1 | 55 |
| Tetra o methyl scutellarein | 342.35 | 3.60 | 0 | 0 | 48 |
| Di alpha tocopherol | 430.72 | 2.06 | 2 | 1 | 110 |
| N,n diacetylurea | 144.13 | -1.53 | 2 | 5 | 80 |
| Glycerol | 92.09 | -1.60 | 3 | 3 | 87 |

**Analysis of the active site of the target ER α**

The active site predictions were done using Meta pocket program. The best ligand binding sites for ER α was observed as follows: MET343, LEU346,THR347, LEU349,ALA350, ASP351, GLU353, LEU354, TRP383, LEU384, LEU387,MET388, LEU391, ARG394, PHE404, VAL418, GLU419, GLY420, MET421, ILEU424, PHE425, LEU428, GLY521, HIS524, LEU525, TYR526, MET528, LYS529, CYS530, LYS531, ASN532, VAL533, VAL534, PRO535, LEU536, LEU539. These binding sites were shown in Fig2 as visualized in Pymol.

**Docking Analysis**

The above predicted active residues were used as the catalytic sites for 7 phenolic compounds in docking studies. The results of the interaction between the active site residues of target protein ER α and 7 phenolic compounds were shown in the Table 2. By analyzing the docking interactions, Guanosine and Tetra o methyl scutellarein were found to have the highest activation energy (greater than -7.5kcal/mol) against ER α than the other compounds. From the docking results, it was finally revealed that among the 7 phenolic compounds, Guanosine and Tetra o methyl scutellarein exhibits the best binding interaction with ER α as shown in the fig 3 & 4 can be further concluded that these two compounds may act as good inhibitors against breast cancer target protein.

**Table 2:** Docking results between ERα and the seven active compounds of *Orthosiphon stamineus*

| S. No | Compounds | Pubchem | Structure | Binding energy (kcal/mol) |
|---|---|---|---|---|
| 1 | Guanosine cyclic monophosphate | 24316 |  | -8.6 |
| 2 | Tetra o methyl scutellarein | 96118 |  | -7.8 |
| 3 | Di alpha tocopherol | 14985 |  | -7.4 |
| 4 | Squalene | 638072 |  | -7.1 |
| 5 | Stigmasterol | 5280794 |  | -6.1 |
| 6 | N,n diacetylurea | 69487 |  | -5.6 |
| 7 | Glycerol | 753 |  | -3.7 |
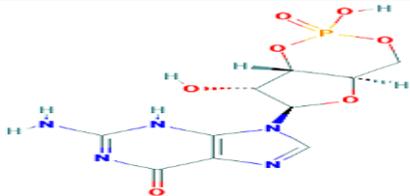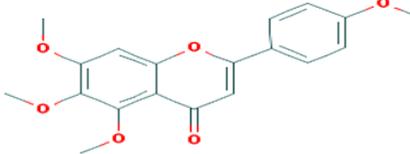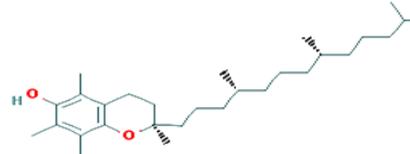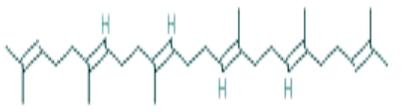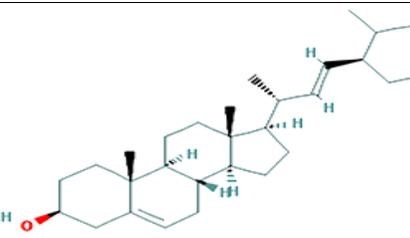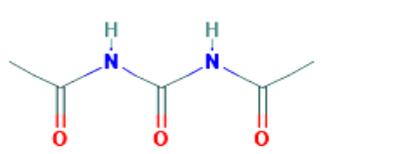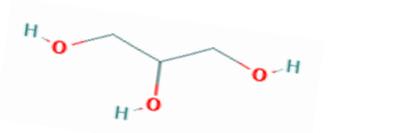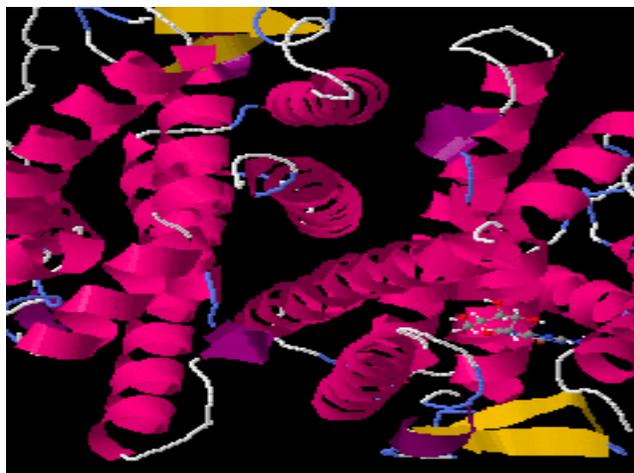
**Fig. 3: Docking between 2IOG and Guanosine were viewed in pymol**



**Fig. 4: Docking between 2IOG and Tetra o methyl scutellarein were viewed in pymol**

## CONCLUSION

In this study, the molecular docking analysis was done to explore the binding mechanism and to correlate its docking score with the activity of compounds. This study concludes that the two compounds namely Guanosine and Tetra o methyl scutellarein when compared to other compounds isolated from *Orthosiphon stamineus* may serve as valuable drug candidates for the treatment of breast cancer. The results of our present study can be useful for the development of novel lead compounds having better inhibitory activity against ER α.

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