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Molecular Docking Studies and Orbital Analysis to Identify Anti-Cancerous Potentials to Prevent Breast Cancer

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ABSTRACT

Breast cancer is the cancerous condition that develops in breast tissues, which could be invasive or non-invasive cancer. Hormonal therapy is general practice to control the estrogen level in Estrogen Receptor – positive (ER+) condition of breast cancer. Phytoestrogens that have been hypothesized to reduce risk of breast cancer and Glycine max (Soya bean) is a primary source of isoflavones, which has structure similar to estrogen receptor molecule. Glycine max intake in the diet has been associated with low risk for developing breast cancer. The aim of the study was to identify anti-cancerous potentials from Glycine max with the help of docking studies and molecular orbital analysis. *Insilico* docking studies were carried out using computational methods, based on Lamarckian genetic algorithm and PM7. This theoretical analysis could lead to further improvement of potent estrogen receptor antagonists for treatment of cancerous conditions.

Keywords: Glycine max, Phytoestrogens, Molecular Docking, Autodock, MOPAC

I. INTRODUCTION

Breast cancer accounts for 22.9% of all cancers in women worldwide [1]. Breast cancer cells have estrogen receptors on their surface and in their cytoplasm and nucleus. Chemical messengers such as hormones bind to receptors, and this causes changes in the cell. Estrogen receptors are over-expressed in around 70% of breast cancer cases, referred to as "ER-positive". Breast cells that are estrogen and progesterone receptor positive (i.e., ER+ and PR+) are more likely to respond hormonal therapy (e.g., Tamoxifen, Raloxifene, Toremifene) and have a better prognosis than cancers that are hormone receptor negative [2]. Natural products have been established to exert anti-cancer activities partially based on their ability to quench reactive oxygen species and protect critical cellular components like DNA, proteins and lipids from oxidative damage [3]. Soybeans are the most common source of isoflavones in human food. Soybeans contain phytoestrogen that is considered by some dieticians and physicians to be useful in the prevention of cancer [4]. Intake of Soy isoflavones is associated with a significant reduced risk of breast cancer in Asian populations [5]. Several experimental studies have shown that soy or isoflavones have anticarcinogenic effects on hormone-related cancers and that these effects may be related to their estrogenic, antiestrogenic, or other activities [6-9]. Interestingly, the chemical structure of Isoflavones is similar to estrogen [10]. The compounds with structural similarity will possess functional similarity.

The use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process [11,12]. There is a wide range of software packages available for the conduct of molecular docking simulations like, Auto Dock, GOLD, and FlexX [13]. Auto Dock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed [13]. In this study, among different phytochemicals, Stigmasterol and Daidzein showed promising binding with estrogen receptor due to the fact that structure of estrogen shares striking resemblance with these phytochemicals. Molecular docking determines the binding affinity between the protein and ligands, which aims to determine the 3D conformation and binding interactions.

II. METHODS AND MATERIAL

A. Ligand preparation

The structures of five phytochemicals namely Campesterol, Genistein, Stigmasterol, Sitosterol and Daidzein (Figure 1) used in this study were retrieved from Pubchem compound database. These phytochemicals satisfied Lipinski's rule of five and ADME properties. The structures were takes as input for docking program in Autodock 4.0.

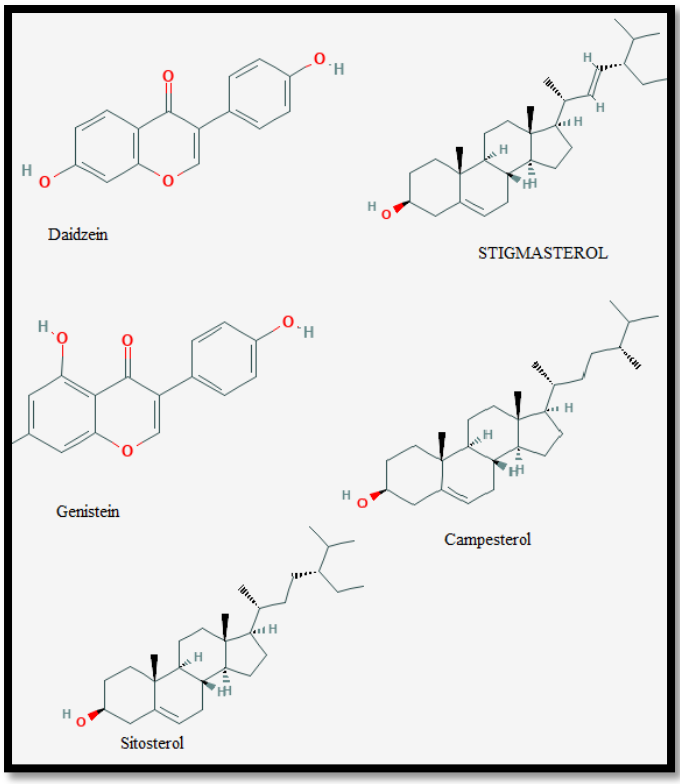

Figure1. Phytochemicals structures present in Glycine max

B. Target Selection

The three dimensional structure of Human estrogen receptor was obtained from the protein data bank (PDB ID: 2IOK, Res: 2.40 A°) [14] (Figure 2). The water molecules were removed during molecular modeling. The energy minimized protein structure was included prior to docking to accommodate hydrogen atoms.

C. Energy minimization

Energy minimization were done to the protein using Swiss PDB viewer to compute energy and energy minimization process [15]

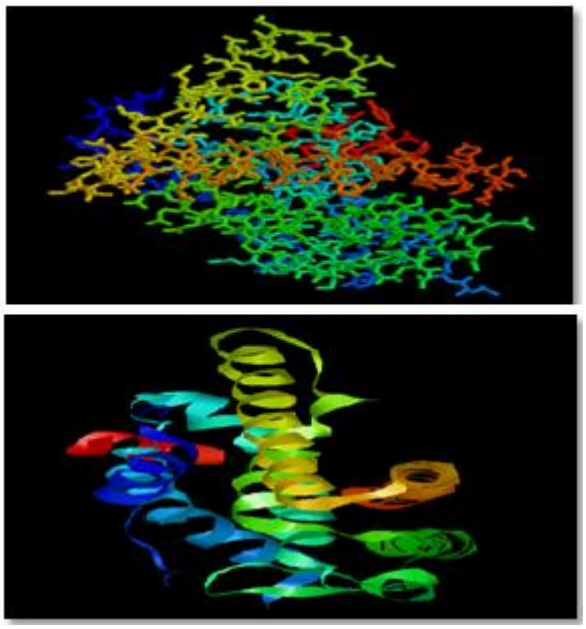

Figure 2. Different schematic representation of 3D structure of Estrogen Receptor (PDB ID: 2IOK)

D. Binding site

The binding site in estrogen receptor was determined using Computer Atlas of Surface Topology of Proteins (CASTp). CASTp helps in identifying geometric properties of protein pockets, which are assumable positions on protein surface. The residues within the binding site were identified. CASTp (Figure 3) calculated potential active sites of protein. The analysis revealed there are several pockets, which fit in role of active site.

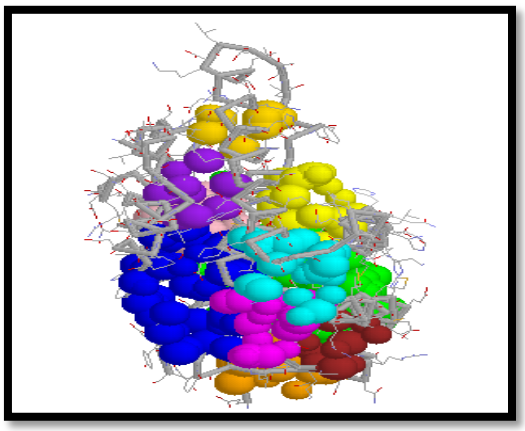

Figure 3. Active sites predicted in the Estrogen Receptormolecule using CASTp server

E. Molecular docking

Molecular docking combined with a scoring function can be used to screen potential drugs *insilico* to identify molecules that are likely to bind to protein target of interest. To perform the docking model, the Auto Dock 4.0 suite molecular-docking tool was used and

methodology was followed [16]. Auto Dock 4.0 is widely distributed molecular docking software which performs flexible docking of ligands into a known protein structure. The default parameters of automatic settings were used. Each docking experiment consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations. The size of grid box is key parameter in Auto Dock. The volume of the box was fixed to 27000Å to have large search space. The Auto Dock results indicated the binding position and bound conformation of protein, as well as hydrogen bond interactions between protein and ligand molecule. The docked conformation, which had minimum binding energy, were selected to analyze mode of binding

F. Molecular orbital analysis

Electronic effects of drug like molecules play an important role in the pharmacological effects [17]. HOMO energy proposes the region of the small molecules, which can donate electron during the complex formation, while LUMO energy signifies the capacity of the molecule to accept the electrons from the target protein. The difference in HOMO and LUMO energy, known as energy gap, indicates the electronic excitation energy [18] that is necessary to compute the molecular reactivity and stability of the compounds [19]. Orbital analysis of compounds were computed using MOPAC2016 [20] and Arguslab [21] softwares. ESP (Electrostatic Potential) mapped density to identify region of electrophilic and nucleophilic attack

III. RESULTS AND DISCUSSION

A. Molecular docking

Molecular docking is key tool in drug modeling process. Docking is a novel method in which the ligand, binds on the pockets or active site of the receptor molecule. This method is regarded as one of the major innovation in drug discovery.

The five phytochemicals were docked using Auto dock 4.2 successfully. The interactions and binding energy of phytochemicals are listed in **Table 1**. Good interactions were observed between residues of protein and ligand molecules. The phytochemicals showed binding energy between -5.50 to -7.50 kcal/ mol. The results were analyzed based on binding energy of the complex. The number of H-bonds was calculated with bond length between atoms of protein-ligand docked complex. Stigmasterol and Daidzein illustrate high affinity for the protein molecules with score of – 7.50 and -7.36 (**Figure 4** and 5**)**. Both phytochemical showed two hydrogen bond interactions with estrogen receptor. Stigmasterol shows interactions with ARG515 residue of protein. Daidzein showed two hydrogen interactions with GLY521 and GLU 353 residues of Estrogen Receptor molecule and bound to active sites.

TABLE 1.
DOCKING RESULTS OF GLYCINE MAX PHYTOCHEMICALS WITH ESTROGEN RECEPTOR PROTEIN

Phytochemicals	**Binding energy kcal/mol**	**Hbonds**	**Protein Ligand interactions (Hbonds)**
Stigmasterol	-7.50	2	LIG 1: O – A:ARG515:HE LIG 1: O – A:ARG 515:HH21
Daidzein	-7.36	2	LIG 1: H – A:GLY521:O LIG 1: H – :GLU353:OE1
Genistein	-7.11	2	LIG 1: H – A:TRP393:NE1 LIG 1: H – A:GLU353:O
Campesterol	-6.80	1	LIG 1: H – A:GLU443:OE1
Sitosterol	-5.70	1	LIG 1: H – A:HIS377:ND1

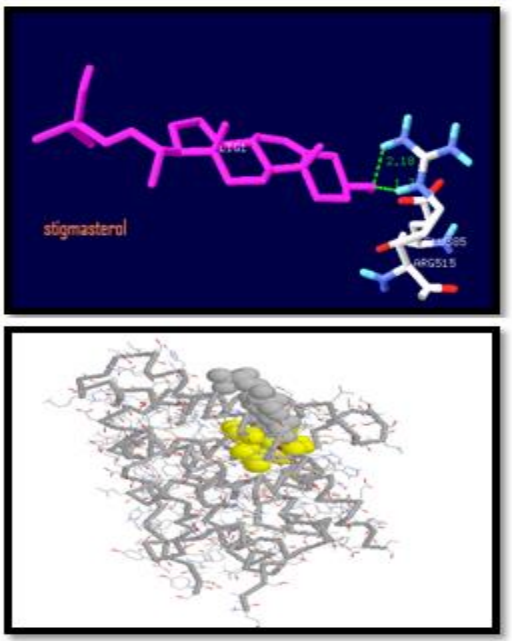

Figure 4: Stigmasterol interacting with residue ARG515 of Estrogen Receptor and bound to the active site of protein molecule

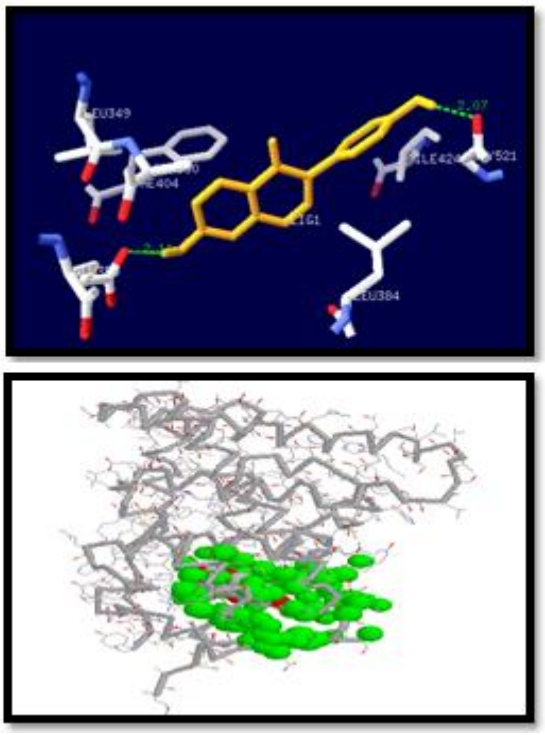

Figure 5: Daidzein interacting with residues GLY521 and GLU353 of Estrogen Receptor and bound to active site of Estrogen Receptor molecule

The screening of phytochemicals from Glycine max against breast cancer target Estrogen Receptor was carried out using molecular docking methods. The docked five compounds demonstrated binding affinity towards estrogen receptor. Among the five, Stigmasterol and Daidzein were identified with least binding energy of -7.50 and -7.36. The molecular docking of the two compounds showed good binding mode and interaction energy. H-bond pattern was analyzed and confirmed inhibition of cancer target and showed that the phytochemicals possessed possible anticancer activity.

B. Molecular orbital analysis

HOMO-LUMO plays an important role in stabilizing the interactions between drug and receptor protein [19]. The orbital energy of HOMO, LUMO and Energy gap between them was calculated to estimate the chemical reactivity of the selected compounds using PM7 (Table 2). Higher values of E_{HOMO}, stronger is electron donating ability of molecule and lower values of E_{LUMO}, more probable it is that molecule would accept electrons [22].

The energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$) is an important quantum chemical parameter that determines molecular electrical transport properties and is a measure of electron conductivity. A molecule with a small energy gap is more polarizable and is generally associated with high chemical reactivity, low kinetic stability and is also termed as a soft molecule [23]. The Energy gap for Daidzein (7.137) was lower compared to Stigmasterol (10.331), so Daidzein are more reactive than the latter. The positive and negative phases of the orbital are represented by the two colors [Figure 6], the blue regions represent an increase in electron density and the red regions a decrease in electron density [24].

TABLE 2.
MOLECULAR PARAMETERS OF STIGMASTEROL AND DAIDZEIN

S. No.	Chemical parameters	Stigmasterol	Daidzein
1.	**Homo eV**	-9.009	-8.652
2.	**Lumo eV**	1.322	-1.515
3.	**Energy gap eV**	10.331	7.137

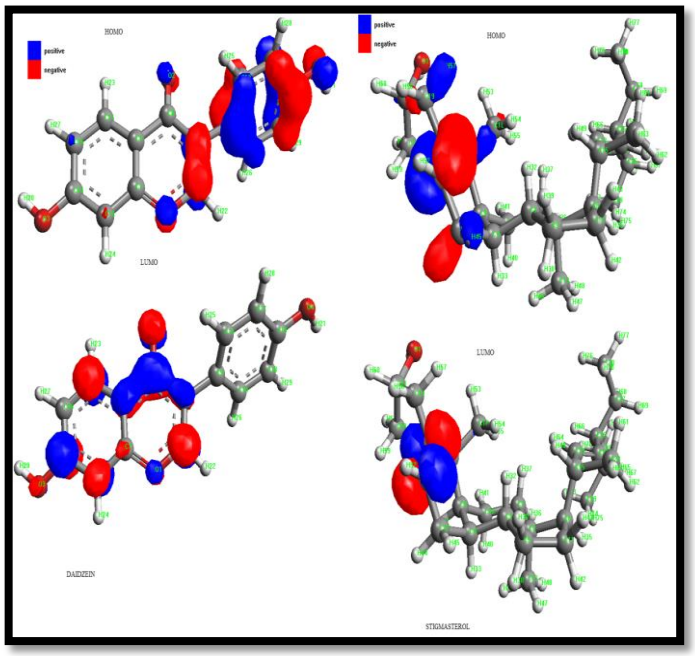

Figure 6. Molecular Orbitals of Daidzein and Stigmasterol

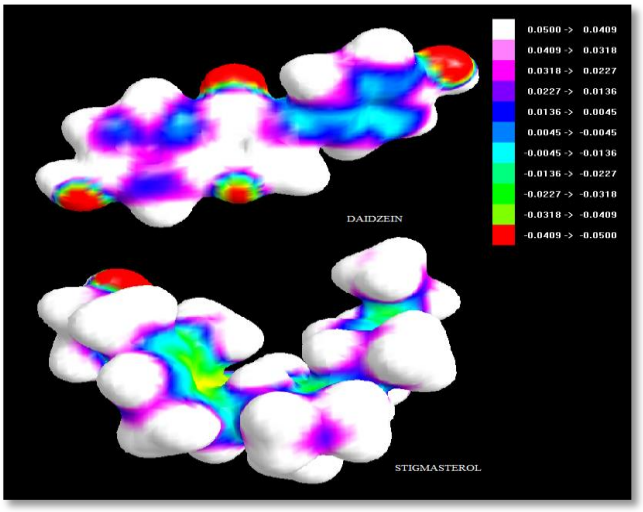

Figure 7. ESP-mapped density of Daidzein and Stigmasterol

ESP-mapped density surface (Figure 7) can be used to show the regions of a molecule that might be more favourable for nucleophilic or electrophilic attack [25]. A positive electric potential means that a positive charge will be repelled in that region of space. A negative electric potential means that a positive charge will be attracted. A portion of a molecule that has a negative electrostatic potential will be susceptible to electrophilic attack – the more negative the better [24].

IV.CONCLUSIONS

The work based on *Insilico* studies showed that Stigmasterol and Daidzein possess better anticancer activity against Estrogen receptor. These compounds are ecofriendly and have fewer side effects. Theoretical analysis would be useful in both understanding triggering effect of Glycine max phytochemicals as well as prove useful in further drug discovery process. From the present study, it can be concluded that Stigmasterol and Daidzein can be used as an Estrogen receptor inhibitor in future. Further *Invivo* studies on these lead compounds can be done to confirm inhibition and used in treatment of Breast cancer.

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