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Short Communication

IN SILICO INTERACTIONS OF DI-HYDROXY MYRICETIN WITH EZH2 PROTEIN

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ABSTRACT

Development of an alternative and complementary method for cancer prevention and treatment can be achieved by discovering effective herbs and elucidating their underlying mechanisms. Myricetin is a naturally occurring flavonol with hydroxyl substitutions at the 3, 5, 7, 3', 4' and 5' positions. We followed simple molecular docking method to screen the interaction between a polycoumb protein EZH2 and dihydroxy myricetin, a flavonol isolated from fruits of *Ficus glomerata Roxb.*Amino acid sequences Gln 791, Asn 842, and Met 793 of prostate cancer protein interacted with isolated flavonoid dihydroxy myricetin. These interactions support the EZH2 molecule to become a lead drug candidate for combating prostate cancer.

Keywords: Prostate cancer, Myricetin, Molecular docking, CADD

1. INTRODUCTION

Prostate cancer has the highest prevalence of any non-skin cancer in the human body and it is the second most leading cause of cancer related death in men [1]. The role of bioinformatics is in progress from the simplest nucleic acid and amino acid to the search of lead drugs using ligands and targets. Properties of ligand are capable of binding to two similar proteins or homologous proteins which can be used for studying the drug like ligands. Further these ligands can be identified on the basis of structure activity relationship [2]. A conventional experiment is practiced for determining efficacy of drug and its safety which requires lot of time and money. Hence researchers are turning towards a standard test for the assessment of drug safety and various approaches to search a new lead compound is under trial [3, 4].

Computer aided drug designing (CADD) has been spectacularly remodeling research and development pathways in search of drug candidate along with rapid raise in biological and chemical information. The process of drug discovery and development and their implementation time and money was eased by computational techniques [5]. Molecular docking is a proficient tool for discovering ideal micro molecule drugs for targeting protein [6].

Docking strategy plays an important part in advanced drug discovery. To enhance the computational speed and accuracy Wang J *et al.,* [7] have immensely contributed to the field of docking research. Due to its role in structure based drug design, protein-ligand docking is gaining high reputation [8, 9].

Molecular docking is basically a computational method. It predicts association of receptor to non covalent macromolecule with a small molecule (ligand) efficiently. The molecular docking, aided prediction of small molecule binding to proteins has a vast influence for the prediction results. These results are used to screen virtual libraries of drugs or drug like molecules to identify lead candidate for drug development. Molecular docking can be used to calculate the conformation of known binders, in absence of the experimental structures [10].

Enhancer of zeste homolog 2 **(**EZH2) as being over expressed in metastatic prostate cancer conditions [11]. In clinically localized prostate cancer, Enhancer of zeste homolog 2was found to be predictive of poor outcome post prostatectomy (*i.e.,* biochemical recurrence or metastasis). Enhancer of zeste homolog 2 is a Polycomb Group (PcG) protein homologous to Drosophila enhancer of zeste and involved in gene silencing. Polycomb Group proteins are presumed to function in controlling the transcriptional memory of a cell. Dysregulation of this gene silencing machinery can lead to cancer [12, 13]. Enhancer of zeste homolog 2 functions as a transcriptional repressor, and inhibition of Enhancer of zeste homolog 2blocks prostate cell growth.

Interestingly, several recent studies have demonstrated that the Enhancer of zeste homolog 2has enzymatic activity and functions as a histone H3 methyl transferase. Biochemical analysis indicates that Polycomb Group proteins belong to at least two multimeric complexes, PRC1 and EED- Enhancer of zeste homolog 2. These complexes are thought to be heritably silence genes by acting at the level of chromatin structure. The EED protein interacts directly with type 1 histone deacetylases (HDACs) in mammalian cells, and this has been suggested to be part of the silencing mechanism [14-17].

2. EXPERIMENTAL PROCEDURE

2.1. Bioactive compound

Fruits of *Ficus glomerata Roxb.* are subjected to soxhlet extraction and methanol extract was centrifuged at 10,000 by rpm for 15minutes; supernatant was used for the purification and structural elucidation as mentioned in previous chapter. Isolated flavonol dihydroxy myricetin (DHM) was used for the experiment in the present study [18].

2.2. Sources and Software

Web sources such as Protein data bank and Drug Bank are used for obtaining the protein and ligand data. Schrodinger software Maestro 9.7 version (Protein prep, lig prep, Glide XP, XP visualizer) was used and Chem Draw 12.0 (Chem Bio finder, 3D Chem Pro) was obtained from online source.

2.3. Selection and Preparation of Protein

Enhancer of zeste homolog 2 (EZH2) is one of the major protein responsible for the progression of prostate cancer cells. Many of the previous studies have been reported on the role of EZH2 in prostate cancer. EZH2 was downloaded and saved in PDB format from protein data bank(PDB) website (www.pdb.org) with PDB ID 4MI5. Most of the structures deposited to PDB are from X-Ray crystallography studies. Hence they lack the necessary protons, side chains, loops, counter ions and bondings. PDB file of 4MI5 was opened in protein preparation wizard application of Schrodinger software. Water molecules with less than 3 H atoms were removed and optimized; pH was adjusted to 6.7 to 7.3. The prepared protein was stored with a new file name [19].

2.4. Ligand Preparation

Di hydroxyl myricetin was isolated and purified from fruits of *Ficus glomerata*. Three dimensional structure of the drug candidate was drawn using the chemdraw 3D applications of chemdraw 12.0 version. To obtain the best results, the structures that are docked must be of good representation of the actual ligand structures. Three dimensional structure was opened with ligand preparation wizard- Ligprep of Schrodinger software and checked for its 3D accuracy, bond length and bond angles, valances and pH. [20].

2.5. Protein-Ligand Docking

Docking process describes a process by which two molecules fit together in three – dimensional space. Prepared protein and ligand was docked using Glide XP software of the Schrodinger software Maestro 9.7. Glide XP software performs docking in two steps: Receptor grid generation and Ligand docking. Receptor grid generation calculates the electrostatic and Vander Waals potentials of binding pockets using grid based methods. Ligand docking generates the confirmations and orientations inside the binding pocket in presence of grid potentials [21].

2.6. Visualization of docking results

Results of the protein ligand study was analysed using XP visualize application of Maestro 9.7 software.

3. RESULTS AND DISCUSSION

The protein responsible for prostate cancer was downloaded from Protein data bank and its three dimensional structure is shown in Fig.1.

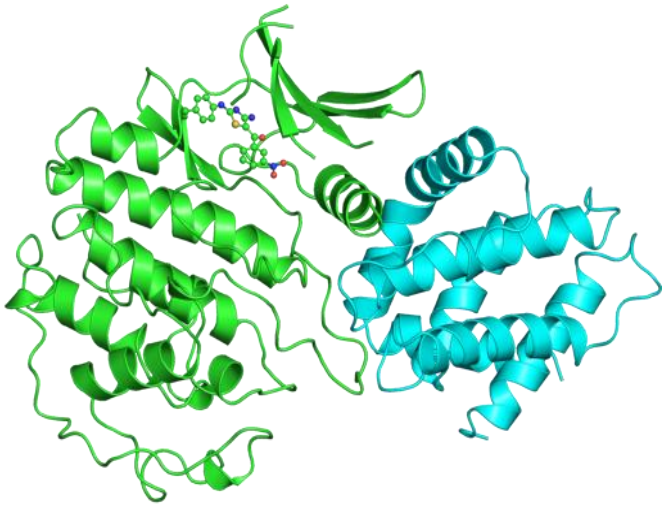

Fig. 1: Showing three dimensional structure of Enhancer of Zeste Homologue 2 protein

Dihydroxy myricetin was isolated from ripe fruits of *Ficus glomerata Roxb.* The spectroscopic studies FTIR, NMR

and LC-MS data reveales the functional group, chemical shifts and mass of the compound. Using these spectral details, the compound was identified as dihydroxy myricetin. Molecular formula and elemental analysis of isolated compound is $C_{15}H_{10}O_{10}$ and C- 51.44, H- 2.88 and O- 45.68.respectively. The molecular weight of the compound is recorded about 353.06.

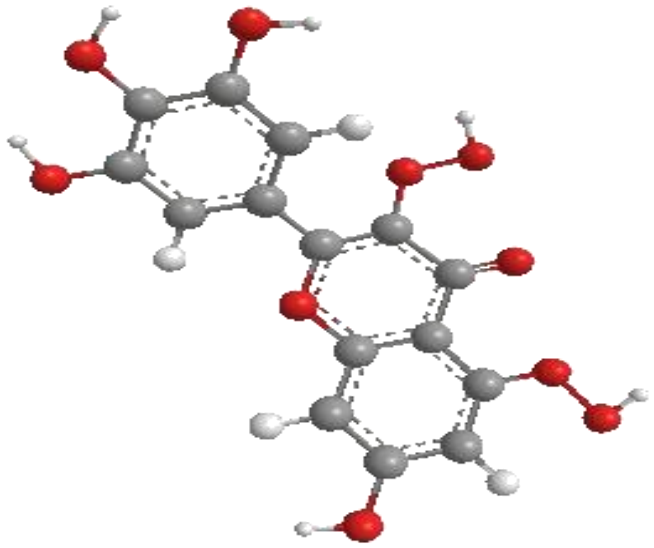

Fig. 2: Three dimensional structure of the isolated flavonoid dihydroxy myricetin

Using GLIDE XP tool protein ligand docking was studied. When ligand interacts with protein of prostate cancer cell, the occurring interaction values were found to be -8.1kcal/mo/ G score, Lipophilic value recorded as -2.71, Bond value of ligand and protein was -4.28 and electrostatic force was -0.65. Amino acid sequences Gln 791, Asn 842, and Met 793 of prostate cancer protein interacted with isolated flavonoid dihydroxy myricetin. These interactions support the EZH2 molecule to become a lead drug candidate for combating prostate cancer (Table 1).

Table 1: Docking scores of the Protein Ligand using GLIDE XP

Parameter	Score
Protein ID	**PDB 4MI5**
Ligand ID	**CID5281612**
G Score kcal/mol	**-8.1**
Liphophilic value	**-2.71**
Bond value	**-4.28**
Electrostatic force	**-0.65**
Protein ligand interaction	**Gln 791, Asn 842, Met 793**

Rapid *in silico* studies are the alternatives to biological assays of primary phases for drug development. Dihydroxy myriceitn was predicted to be a potential drug candidate against prostate cancer because of the antineoplastic activity of flavonoids and displaying drug like properties. EZH2 protein has 1091amino acid of single poly peptide chain. The amino acid Glycine 791, Asparagine 842 and Methionine 793 of EZH2 have shown possible interactions.

Studies of Azhaguraj *et al.,* [5] has indicated the antitumor properties of drugs isolated from algae using computer aided drug design which has possible interactions between protein and ligand. Hence from preliminary insilico screening of flavonoid against EZH2 protein that supports its suitability for a lead compound to carry out further Insilico studies to understand its pharmacokinetic properties.

Isolated flavonoid dihydroxy myricetin is screened for its anticancer properties through insilico studies. Enhancer of zeste homologue 2, protein which is highly expressed in prostate cancer cells was docked with isolated flavonoid to study the possible interaction between ligand and protein.

Dihydroxy myricetin has shown acceptable anticancer studies under under *insilico* conditions dihydroxy myricetin has shown possible interactions with enhancer of zest homologue 2 a protein of prostate cancer cellat three different amino acid positions.

4. CONCLUSION

Interaction of dihydroxy myricetin with enhancer of zest homologue 2 protein of prostate cancer cells under *insilico* conditions. Hence, it is fair to conclude that the *insilico* screening and analysis methods carried out in the present research work has provided hope to profile the lead drug compound dihydroxy myricetin from fruits *Ficus glomerata* which can provide new avenues in treating prostate cancer.

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