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Phylogenetic Analysis of Dietary Flavonoids Against COX-2 Protein Causing Colon Cancer

Meena Sharma[1], Shallu Kalia[2]

Department of Botany, Hans Raj Mahila Maha Vidyalaya , Jalandhar-144008, Punjab, India

Department of Bioinformatics, Hans Raj Mahila Maha Vidyalaya , Jalandhar-144008, Punjab, India

Abstract: ***Flavonoids are a class of polyphenols found in fruits, legumes, vegetables, nuts, seeds, grains and tea. Citrus flavonoids are found in fruit tissue, juice, pulp and stem. Naringin of grapefruit and hasperidin of orange rarely occur in other plants and unique to these fruits. Power of flavonoids is dazzling. They are antioxidant, antimutagenic, antiallergic, neuroprotective and also have been shown to inhibit cancer cell growth. Increased cyclooxygenase activity promotes progression of colorectal cancer. In this research report, phylogenetic relationships of various flavonoid plant sources such as Apricot(Prunus armeniaca), Strawberry(Fragaria x ananassa), Rapeseed (Brassica napus) , Apple (Malus Domestica) , Grapes (Vitis vinifera) , Tomatoes (Solanum lycopersicum) , Onions (Allium cepa), Soyabean (Glycine max), Mulberry (Morus alba),Potato(Solanum tuberosum), Cocoa (Theobroma cacao), (Citrus clementina) has been done with the use of CLUSTAL-omega and PYLIP. It is also found with the help of literature that mainly Grapes contain high flavonoid content due to the presence of MYB genes in Vitacea family than the other species of the Vitacea family. It is seen that no genome wide characterization has been done on these species. Increased cyclo-oxygenase activity promotes progression of colorectal cancer. This study is therefore aimed to target COX-2 as target protein and Grape Flavonoids as ligand to prevent colon cancer. Colon cancer is the third most common cancer among all the countries especially in western ones after the Breast cancer and Lung cancer in either sex. In India, Colon cancer is sixth in number in a deadly disease position. In the present research report, dietry flavonoids have been used as nutraceutical that provides health and medical benefits, including the prevention and treatment of disease. In this way, flavonoids are playing a very important role in colon cancer prevention. In the present report, molecular docking of COX-2 has been done as a target protein with the Grape Flavonoids and its analogues with the help of software named as AUTODOCK- 4. Afterwards, ADMET properties of all ligands are measured with ORISIS property explorer software which is freely accessible.***

Keywords: Flavonoids, Cyclooxygenase, COX-2, MYB genes, prostaglandin E2 (PGE2)

1. Introduction

Flavonoids are a very large and diverse group of phytonutrients (more than 6000 have been so far identified). Five sub categories of dietary flavonoids are:, Flavonols, Flavon-3-ol, Flavones, Flavonones and Anthocyanidins present in onion, apple, tomato, orange, grapes, blueberries, banana, brassica etc. They are best known for their anti-oxidant, anti-inflammatory and anti-cancerous health benefits. Cancer is increasing day by day and takes lives of millions or billions of people in number. Everyday billions of cells get destroyed and error in the repair of these cells lead to mistakes that allows the newly made cell to multiply in an uncontrolled manner, independent of the checks and balances that control normal cell growth. When this happens, a mass of abnormal cells (that is Tumor) can develop [12]. Cancers are named by the tissues from which the first tumor arises. It is found by the various early researchers that each cancer is a different disease with varying prognoses (likely outcomes) and different treatment options. It is essential to treat each person with a diagnosis of cancer as an individual regardless of the type of cancer. Here, in this research report, the colon cancer is taken as disease because it is the sixth in position in India among the deadly disease list and almost second or third in number in US and other western ones. This created interest to find a target protein in colon cancer so as to treat the patient with effective drug rather than the side-effective therapies. To treat colon cancer, the use of Nutraceuticals (Vitis Vinifera-flavonoid content) is taken as ligand [11]. COX-2 plays a very imperative role in colon cancer. Cyclooxygenase is a key enzyme in the prostanoid biosynthetic pathway [14]. It has received considerable attention due to its role in human cancers. COX 2, is undetectable in most normal tissues and is induced by proinflammatory and mitogenic [15]. There is extensive evidence, beyond the finding that COX 2 is commonly overexpressed in tumors, to suggest that COX 2 is mechanistically linked to the development of cancer [7]. The most specific data supporting a cause-effect relation between overexpression of COX 2 and carcinogenesis come from genetic studies. COX-2, the inducible isoform of cyclooxygenase plays a bona fide role as pharmalogical target for cancer prevention [14]. COX 2 affects many processes that are important in carcinogenesis, which makes it an attractive therapeutic target than the other proteins [16]. These include xenobiotic metabolism, angiogenesis, apoptosis, inflammation, and immunosuppression.

2. Materials and Methods

NCBI: For obtaining sequences of flavonoid containing plants in fasta format.

CLUSTAL-Omega: is software used to find multiple sequence alignment of the given NCBI sequences.txt file of various flavonoids containing sources .Save the result in PHYLIP format.

PHYLIP: is used for Phylogenetic analysis and tree construction. Species from various flavonoid containing sources are aligned in PHYLIP format. This is mainly phylogenetic relationship study in different flavonoid sources

using neighbor join method with the help of Clustal -Omega and Phylip software.

Protein Data Bank: commonly known as PDB, is used in the study to find the protein target file that is 1CX2 for COX-2 protein. There are four chains that are A, B, C, and D in 1CX2 PDB file. It is homopolymer protein file. Therefore, any one of chain is selected for further purpose of docking. The function of PTGS2 or COX-2 is that it Converts arachidonate to prostaglandin H2 (PGH2), a committed step in prostanoid synthesis. It is constitutively expressed in some tissues in physiological conditions, such as the endothelium, kidney and brain, and in pathological conditions, such as in cancer. PTGS2 is responsible for production of inflammatory prostaglandins. In cancer cells, PTGS2 is a key step in the production of prostaglandin E2 (PGE2), which plays important roles in modulating motility, proliferation and resistance to apoptosis.

Active Site Finder: is used to find the active site residues of protein text file 1CX2.The PDB file is uploaded in the active site finder software and as a result, a file named "d2" is found. It aid in finding the active site residues. With the help of literature, some of the residues are matched with the interacting active site residues of COX-2. For complete analysis of interacting residues, the below given bioinformatics tool is used.

CAST-p: represented as Computer Atlas of Surface Topology of Proteins, is a tool in Bioinformatics which is an online resource for identifying functional regions and active site of proteins. In this research study, this tool is used for finding the active site residues that were found in the literature. This tool helps very much in finding the active site residues.

Zinc Database: is online software used to find ligand analogues. The files is saved in notepad as Ligand.mol file.

AUTODOCK-4: (ADT) is the main freely accessible software available for molecular docking. This is the best software for docking.

ORISIS property explorer: is used in the present report to analyse the ADMET properties of the ligand and its analogues.

3. Results and Findings

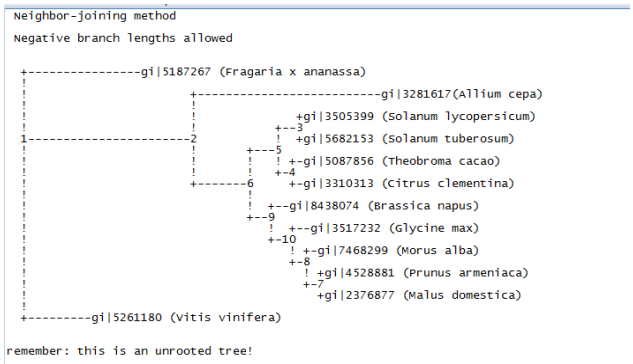

Figure 1: Phylogenetic tree showing flavonoid containing plant species (from literature).

With the phylogenetic tree construction using Phylip (Neighbour Joining Method), it is found that ***Vitis Vinifera*** contains the high flavonoid content due to presence of MYB genes in such species. This is also verified through literature. There are four different files of Ligand Preparation and a single ligand file of flavonoid. With Autodock mgl tool, docking has been done. Autodock finds the energy and displayed interactions. The best overall vina energy found is -7.1 and -7.0. It is also found that analogues docking result is better than original ligand docking result. The interactive active site residue of the protein molecule and best interaction is dislayed below showing all the active site residues:

Protein	PDB-ID	Active site residues
COX-2	1CX2.pdb	LEU 359, TYR 355, ARG120, LEU 352, VAL 523, HIS 90

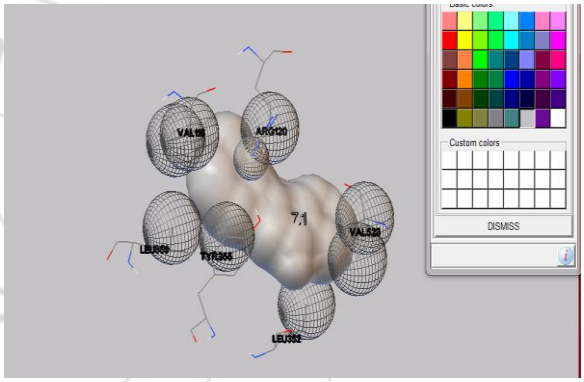

Figure 2: Active sites of Interaction for protein and original Ligand.

The ADMET property is also found at the end of the research to check the Drug properties. It is found that an ADMET property of analogues of ligand is better than its ligand (flavonoid). In the below figure, it could be find that the original ligand is mutagenic .On the flipside, the analogue 4'-Hydroxyflavone shows a significant result showing better Drug properties.

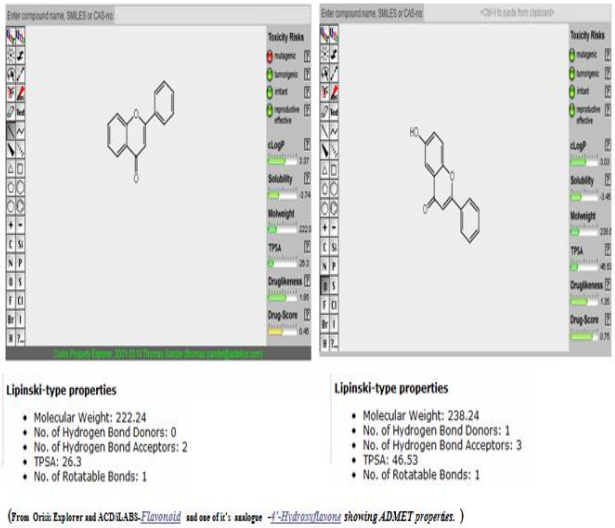

Figure 3: ADMET Properties of ligand and it's one of analogue

4. Conclusion

It is also found that high content of flavonoids is found mainly in *Vitis Vinifera* than the other members of the Vitacea family. The phylogenetic tree construction of flavonoid containing sources has been done using the Neighbor Joining method in Phylip software showing the evolutionary relatedness among these plant species and high flavonoid content in grapes due to presence of MYB genes.

It can be concluded that flavonoid content of the Vitis Vinifera (nutraceutical) could be a potent anticancer target molecule against COX-2 which may be worth for further clinical trials. In this research report, it is looked at how nutraceutical flavonoids act as ligand and could be docked into a protein molecule (PDB ID: 1CX2). COX-2 protein was successfully docked into protein molecule in this study. To increase the efficacy of nutraceuticals, synthesis of analogues has been done. With the molecular target of nutraceutical being known (COX-2), and proper interaction of ligand-receptor, show it possible to develop more refined drug that specifically target it's binding sites. The overall best vina energy found is -7.0 AND -7.1. Active site interacting residues in ligand-receptor (close in contact) found are ARG120, LEU 359, HIS 90, LEU 352, and TYR 355.

In research report it is cleared that analogues of ligand (-7.1) have lower binding energy in comparative to original ligand (-6.9), so they can be better drug like molecules. In future, wetlab work could be done to get authenticate results. Therefore, full exploitation of Nutraceuticals is not yet taken place. There should be proper exploitation of Nutraceuticals to do accurate research.

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