Study of the Effect of Plant Phytochemicals as Cdk-4 Inhibitor for the treatment of Retinoblastoma: An In-silico Approach

Mohana Priya I[1], Azar Zochedh A S[2*], Karthick A[3]

[1]PG Student, Department of Bioinformatics, School of Distance Education, Bharathiar University, Coimbatore, Tamilnadu, India
[2]Research Scholar, Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil, Tamilnadu, India
[3]M.Tech Biotechnology, Sole Proprietor, Madurai, Tamilnadu, India

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Abstract - *Retinoblastoma (RB) is the most common pediatric cancer of eye with an incidence of 1 in 15000 live births and accounts for 3% of all pediatric cancers. RB1, a tumor suppressor gene, encodes retinoblastoma protein (pRB) that regulates various cellular processes such as cell proliferation, differentiation, and apoptosis, and its inactivation results in uncontrollable cell proliferation. Mutations in RB1 gene leading to non-functional or loss of protein are reported in many human cancers including retinoblastoma. Due to involvement of Cyclin Dependent Kinases (Cdks) in the phosphorylation step, its inhibition leads to the activation of the suppressor activity of RB protein that prevents tumour progression. In this present study, we analyze the molecular docking studies of plant derived phytochemicals Naringin, Syringic acid and Berberine hydrochloride to find the binding efficiency of CDK-4 protein. The 3-dimensional structure of target protein is retrieved from the RCSB protein data bank and autodock vina tool helps in molecular docking studies. Docking analysis reveals that among three compounds Naringin shows good binding affinity of -7.7Kcal/mol with the active site of the CDK-4 protein and can be used as a potential drug to target retinoblastoma.*

Key Words: Retinoblastoma, CDK-4, Naringin, Syringic Acid, Berberine Hydrochloride, Molecular docking

1. INTRODUCTION

1.1 Retinoblastoma

Retinoblastoma (RB) is the most common intraocular tumour arising from cone precursor cell with an incidence of 1 in 15000 live births [1]. It is estimated that India alone diagnoses 1500 new retinoblastoma cases every year [2]. Retinoblastoma is genetically characterized by mutation of both alleles of RB1gene and inherited in an autosomal dominant pattern. Totally, 32 clinical signs were reported for the presence of RB. Leukocoria (white reflex) and strabismus are the most common presenting signs in more than 90% of RB cases. Other presentation signs include proptosis, red eye, orbital cellulitis, intraocular hemorrhage and reduction in vision. Retinoblastoma occurs as heritable or non – heritable with one eye (unilateral) or both eyes (bilateral) affected [3]. In 1971, Knudson gave a hypothesis called two hit hypothesis that heritable retinoblastoma, first hit is a germ line mutation and the second hit occurs in retinal progenitor cells (Fig -2). Whereas non – heritable retinoblastoma, both hits occur in retinal progenitor cells only [4].

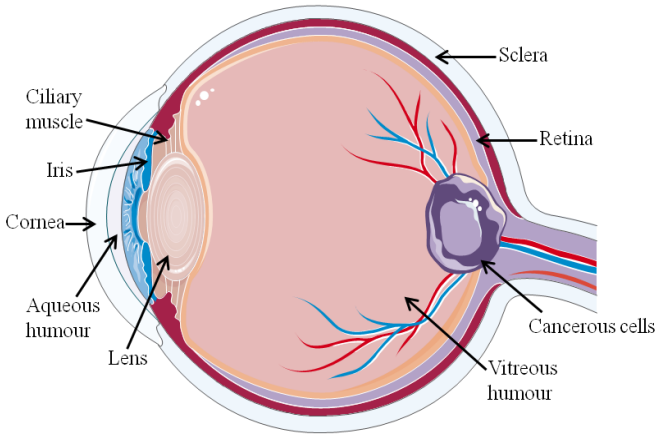

Fig -1: Anatomy of Retinoblastoma eye

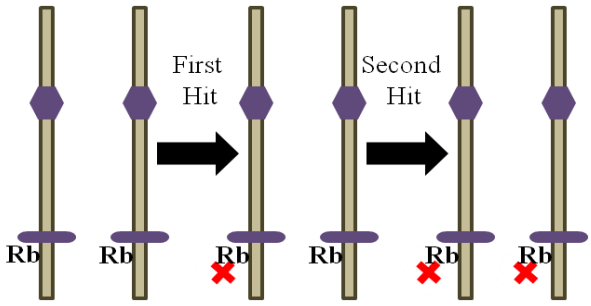

Fig -2: Sporadic RB - Two hit required

1.2 *RB1* Gene

RB1 gene is a tumour suppressor gene, located on the long arm of chromosome 13 in human. The retinoblastoma protein (pRB) regulates the cell cycle by targeting E2F transcription factor and function as transcriptional repressor. RB protein regulates various cellular processes such as cell proliferation, differentiation and apoptosis, and its inactivation results in uncontrollable cell proliferation [5]. Due to involvement of Cyclin Dependent Kinases (Cdks) in the phosphorylation step, its inhibition leads to the activation of the suppressor activity of RB protein that

prevents tumour progression. According to the previous study, it has been reported that CDK-4 plays a vital role in the phosphorylation of pRB and emerged as a planned focus to treat RB [6, 7].

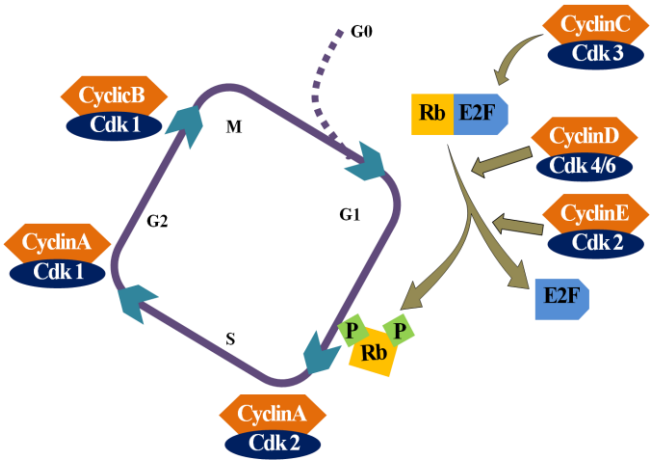

Fig -3: Function of Retinoblastoma protein

1.3 Naringin

Naringin is a flavanone glycoside compound present in grapes and citrus fruits and possesses a distinct bitter taste of grape juice and has strong antioxidant property [8]. The molecular weight of naringin is 80.4g/mol. The molecular and chemical formula are C27H32O14 and 4',5,7,-trihydroxyflavonone-7-rhamnoglucoside respectively [9,10]. Previous literature surveys have reported that naringin has a variety of pharmacological activity such as anti-cancer, anti-inflammation, anti-oxidation, anti-ulcer, anti-mutation, anti-osteoporotic and analgesic activities [11,12]. In addition, naringin can inhibit the excessive activation of the PI3K/AKT/mTOR signalling pathway which results in inhibiting biological function of tumors [13]. In recent survey, it was identified that PI3K/AKT/mTOR signalling pathway as a key target for tumor-targeted therapy [14-17]. These signalling pathway play a vital role in regulating proliferation, migration, growth and tumor cell survival [18,19]. Naringin is reported to show anticancer activity by enacting apoptotic pathway and promotes cell death in tumor cells to prevent the tumor growth with less adverse toxicity to normal cells [20].

1.4 Syringic Acid

Syringic acid is a naturally occurring phenolic compound distributed in wide variety of plant products (fruits and vegetables) as olives, spices, grapes, honey, pumpkin, red wine and certain fungal species [21-23]. The chemical formula and molar mass of Syringic acid are C9H10O5 and 198.17 g/mol respectively. It is soluble in methanol or ethanol and is slightly soluble in water. The other names of Syringic acid are Cedar acid; 4-Hydroxy-3, 5-di methoxybenzoic acid; Gallic acid; 3, 5-dimethyl ether; ; 3, 5-Dimethoxy-4-hydroxybenzoic acid [24]. A previous report shows that, Syringic acid exhibits various properties in biomedical field such as anti-diabetic, anti-microbial, anti-cancer, anti-inflammation and also in prevention of brain/CNS, heart and liver [25]. Syringic acid also plays a major role in the industry sector, acts an effective substrate for fungal laccase enzyme, which has more importance in pulp industry and bioremediation [26]. Syringic acid derived from Tamarix aucheriana shows chemo sensitizing and anti-mitogenic activity in human colorectal cancer cells by involving in the mechanism of apoptosis regulation, cell-cycle arrest, NF κB- DNA binding, cell migration and proteasome activity [27]. Furthermore, literature reported that Syringic acid shows anti-proliferative activity against human K562 leukaemia cells [28].

1.5 Berberine Hydrochloride

Berberine hydrochloride is an isoquinoline alkaloid that has diverse pharmacological activity. It is derived from variety of Chinese herbs such as *Phellodendron chinense schneid, Phellodendron amurense* and *Coptidis rhizome.* It has anti-diabetic, anti-atherosclerotic action, anti-lipid per oxidation activity and also exhibits neuro-protective properties and enhance polycystic ovary syndrome [29-33]. It is widely used as an anti-fungal, anti-bacterial, and anti-inflammatory drug [34,35]. In various cancer cell lines berberine hydrochloride shows anti-proliferative activity that led to further research interest in this compound [36-45]. Berberine hydrochloride exhibits anti-neoplastic properties that induce apoptosis and cell cycle arrest and also inhibit cell migration and invasion through regulation of different pathways [46-49].

Computational biology strategies help to investigate the interaction between the therapeutic drugs and protein. This computer based study saves time and energy and also cost effective. The present study aims to characterize the physiochemical properties of target protein i.e., CDK-4 protein and to evaluate its binding affinity with naringin, syringic acid and berberine hydrochloride through molecular docking.

2. MATERIALS AND METHODS

2.1 Preparation of the 3-dimensional structure of protein

The 3D model of protein CDK-4 was retrieved from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/), (PDB ID: 2w9z) was utilized as a potential drug target for retinoblastoma. The molecule structure of target protein was retrieved in ".pdb" format.

2.2 Ligand identification

Naringin, Syringic acid and Berberine hydrochloride (PubChem ID: 442428, 10742 and 12456) were compounds used for molecular docking study and were obtained from phytochemicals of various plants. These ligand molecules

were retrieved in 3-dimensional structure in ".sdf" format from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

2.3 Physiochemical characterization of the protein model

The protein sequence in FASTA format was obtained from UniProt (UniProt ID: P24385) (http://www.uniprot.org). The obtained FASTA sequence was uploaded in Expasy's ProtParam Proteomics server to analyze physical and chemical parameters of the target protein (http://web.expasy.org/protparam). The molecular weight, amino acid length, pI, extinction coefficient, positive and negative charged residues, grand average of hydropathicity index (GRAVY), instability and aliphatic index of the target protein has been identified.

2.4 Molecular docking through AutoDock vina

The molecular docking was performed using freely available software AutoDock Vina. The target protein was loaded in ".pdb" format and was prepared by adding hydrogen polar atoms and kollman charges and deleting water molecules. The prepared protein was finally saved in ".pdbqt" format. After preparing target protein the ligand molecules were imported in ".sdf" format and were converted to ".pdbqt" format. Then docking region was selected by forming a grid box. After that AutoDock Vina was executed using command prompt and results were analyzed

2.5 Construction of protein-ligand complex

The complex structure of target protein and ligand was built using PyMol 2.4 tool (https://pymol.org/2/). Both the target protein CDK-4 and ligand molecule were imported from the docking workspace. The imported file was in the ".pdbqt" format. Then the construction of protein-ligand complex was made and was saved in ".pdb" format.

2.6 Structure visualization

The protein-ligand complex was visualized using BIOVIA Discovery studio tool 2020. The constructed protein-ligand complex was imported on the graphical window in ".pdb" format. After loading the complex structure charges were added. The complex molecule showed the interaction of amino acid between target protein and ligand in 2-dimension and 3-dimension.Through 2D and 3D structure the hydrogen and hydrophobic bonds were analyzed.

3. RESULTS AND DISCUSSION

3.1 Physiochemical properties of target protein

The Expasy's ProtParam tool was used to analyze the physiochemical characteristics of target protein CDK-4. The result is given in table 1.

Table -1: Physiochemical properties of target protein

Target Protein: CDK-4 (PDB ID: 2w9z)	
Length	295
Molecular weight	33729.11
pI	4.97
-R	47
+R	34
Extinction co-efficient at 280nm	20690
Instability index	57.71
Aliphatic index	92.92
GRAVY	-0.185

The isoelectric point (pI) value calculated for the target protein was less than 7 that shows the acidic characteristic of CDK-4 protein. The molecular weight of protein was 33729.11 Da. The extinction coefficient was calculated at 280nm using the extent of light being absorbed by the protein of target at wavelength range of 20690 $M^{-1}cm^{-1}$. -R and +R denotes the negative (ASP+GLU) and positive (ARG+LYS) charged residues. There are 47negative charged residues and 34 positive charged residues. Based on the Expasy's ProtParam instability index of CDK-4 was 57.71. The grand average hydropathicity (GRAVY) index was negative value of -0.185 demonstrate the hydrophilic nature of the target protein.

3.2 Docking scores of target protein with plant phytochemicals

Further the docking was performed for protein CDK-4 against the plant derived phytochemicals naringin, syringic acid and berberine hydrochloride.

Table -2: AutoDock Vina results of Naringin, Syringic acid and Berberine hydrochloride against CDK-4

Ligand Molecules	Binding Affinity (kcal/mol)
Naringin	-7.7
Syringic acid	-5.9
Berberine hydrochloride	-6.9

The protein of target CDK-4 was docked on the binding pocket with the plant derived phytochemicals naringin, syringic acid and berberine hydrochloride. Based on the lowest energy value (DGbind) and negative value, the best docking orientation was selected. According to the AutoDock Vina result it was analyzed that syringic acid (-5.9kcal/mol) and berberine hydrochloride (-6.9kcal/mol) showed less binding affinity compared to naringin. Naringin shows strong interaction at the active sites and best docking confirmation towards the CDK-4 protein with docking score of -7.7 kcal/mol.

3.3 Analysis of 2D and 3D interaction of target protein with ligands

Our target protein CDK-4 with potent drug naringin formed four (4) Conventional Hydrogen bond interactions with Arg26 (2) and His68 (2) at bond distances 2.11196 Å, 2.77127 Å, 2.47646 Å, 2.20607 Å and one (1) Carbon Hydrogen bond with Phe130 at distance 3.68969 Å.

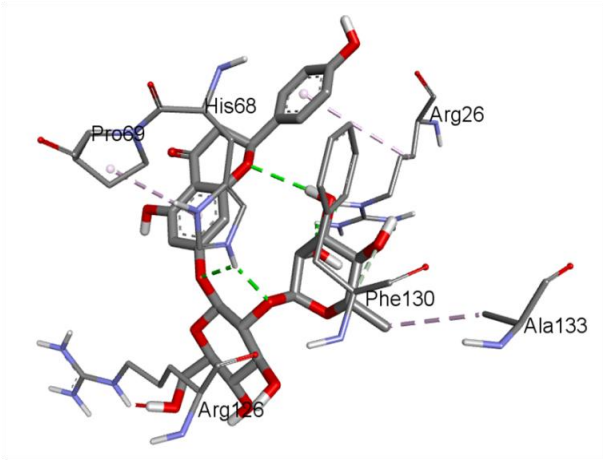

Fig -4: 3D Interaction of Naringin with CDK-4

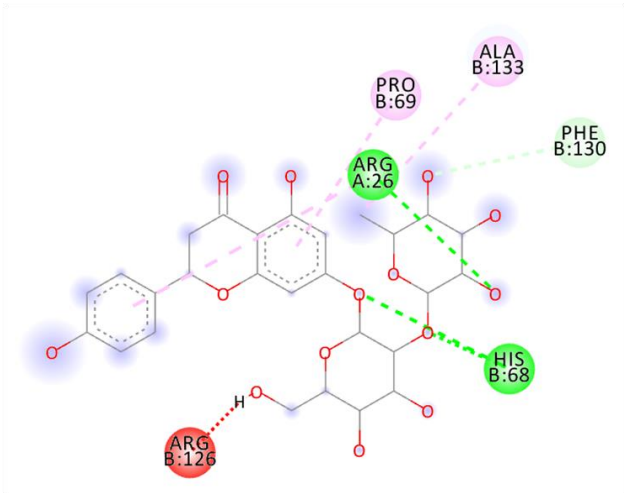

Fig -5: 2D Interaction of Naringin with CDK-4

Also CDK-4 with naringin, whereas alkyl type of hydrophobic interaction was formed at Ala133 with a distance 4.07757 Å and also formed two (2) Pi-Alkyl interactions with Pro69 and Arg26 at bond distances 5.12332 Å and 4.87918 Å respectively. Other interactions include unfavourable donar-donar interaction with Arg126 at distance 2.80802 Å. Thus, the compound naringin was found to be deeply buried in the active site of the ATP binding domain.

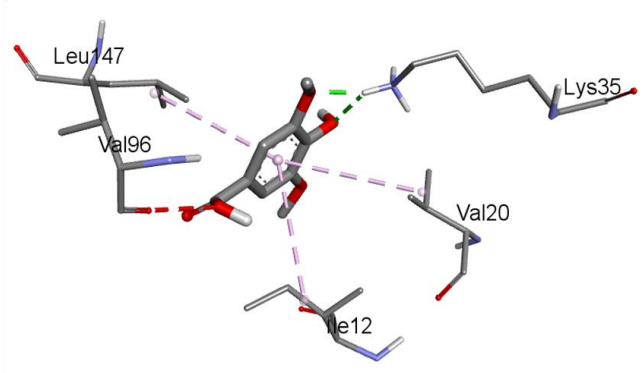

Fig -6: 3D Interaction of Syringic acid with CDK-4

Syringic acid with target protein CDK-4 formed two (2) Conventional Hydrogen bond interactions with Lys35 (2) at distance 1.96122 Å and 2.37577 Å respectively. Also syringic acid with target protein formed three (3) hydrophobic such as Pi-Alkyl interactions with amino acid residues of Ile12, Val20 and Leu147 at 5.03755 Å, 5.14824 Å and 5.04707 Å bond distances. In addition, syringic acid with CDK-4 formed unfavourable acceptor-acceptor interaction with Val96 amino acid residue.

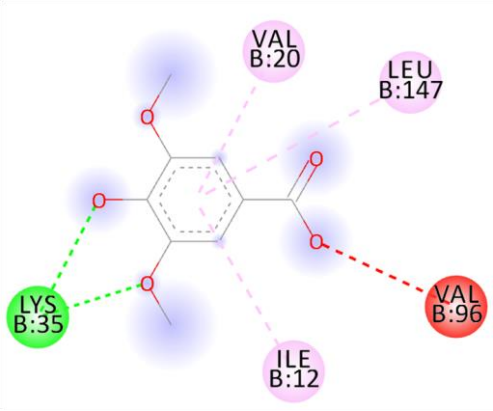

Fig -7: 2D Interaction of Syringic acid with CDK-4

Berberine hydrochloride with target protein formed one (1) Conventional Hydrogen bond interaction with amino acid residue Arg163 at bond distance of 3.05627 Å and one (1) Carbon Hydrogen bond interaction with Glu56 at distance 3.74346 Å.

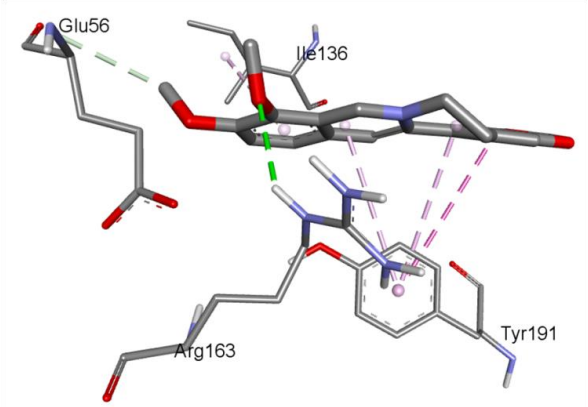

Fig -8: 3D Interaction of Berberine hydrochloride with CDK-4

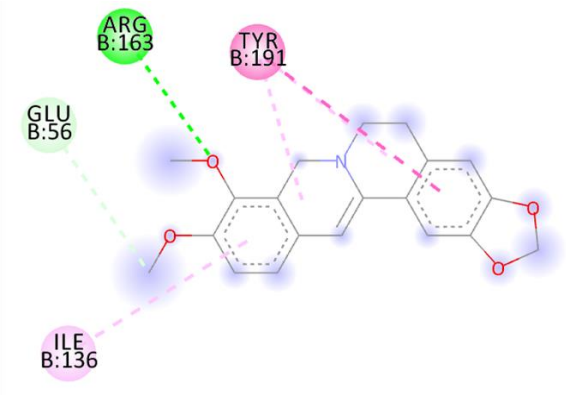

Fig -9: 2D Interaction of Berberine hydrochloride with CDK-4

Whereas, the amino residues Tyr191 (3) and Ile 136 formed one (1) Pi-Pi type and three (3) Pi-Alkyl type of hydrophobic interaction at 5.67776 Å, 5.29779 Å, 5.25385 Å and 5.34719 Å respectively.

Most of the docking studies have reported that binding affinity, number hydrogen bond interaction and bond distances play a major role on influencing protein-ligand interaction [50]. It shows that all the three ligand molecules exhibit good affinity towards the ATP binding site of CDK-4 protein. This could be easily interpreted based on the binding affinity with the CDK-4 protein makes naringin more potent drug than syringic acid and berberine hydrochloride.

4. CONCLUSION

In this study, with the help of molecular docking we have effectively elucidated the effect of plant derived phytochemicals naringin, syringic acid and berberine hydrochloride on the ATP binding site of CDK-4 protein. Among three ligands naringin shows higher binding affinity with CDK-4 with docking score of -7.7kcal/mol. The study can be useful to discover novel inhibitors and to design and develop drugs by validating in-vitro and in-vivo targeting retinoblastoma protein for the treatment of retinoblastoma.

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