RESEARCH ARTICLE

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Targeting Oral Cancer: *In Silico* Docking Studies of Phytochemicals on Oncogenic Molecular Markers

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Abstract

Objective: Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. Oral carcinogenesis is a complex, multistep process in which genetic events within signal transduction pathways governing normal cellular physiology are quantitatively or qualitatively altered. There are various molecular targets like Cyclin D and PI3k- alpha Ras Binding Domain receptor protein involved in the pathogenesis of Oral Squamous Cell Carcinoma. The aim of the study is to demonstrate the computer aided drug design to identify a potent natural molecule for targeting cyclin D4 and PI3K RAS binding protein. **Materials and Methods:** Target selection (Cyclin D1 and PI3K-alpha Ras Binding Domain receptor) was done and structures were derived from protein data bank. Ligands (Apigenin, Chrysoeriol and Luteolin) selection was done and structure derived. Final docking was performed by Autodock.**Results:** From the docking results it can be seen that luteolin has the highest binding energy (-5.45) with the Cyclin D receptor molecule followed by Chrysoeriol (-4.99) and Apigenin (-4.96). The binding energies of the ligands against PI3K-alpha Ras Binding Domain receptors were Apigenin (-4.51), Chrysoeriol (-4.6) and Luteolin (-4.56). **Conclusion:** The study concludes that all the three selected ligands possess high binding energy with both the target proteins involved in carcinogenesis with highest binding energy possessed by Luteolin against the Cyclin D receptor and by Chrysoeriol against PI3K-RAS binding protein. Thus their activity can be utilized to derive potential Anti-cancer therapeutic drugs.

Keywords: Molecular docking- targeted drug- cancer treatment- herbal drugs

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Introduction

Oral carcinogenesis is a multifaceted, intricate process whereby genetic events within signal transduction pathways that regulate regular cellular physiology are modified either qualitatively or quantitatively [1]. Oral squamous cell carcinomas (OSCC), the most common kind of oral cancer, account for up to 80-90% of all malignant neoplasms of the oral cavity. The National Cancer Institute (NCI) has published epidemiological data indicating that the overall 5-year survival rate for OCSCC is 63%, ranging from 83% in the early stages to 38% in the late stages. In oral cancer, certain molecular targets have been identified as significant in the progression of the disease. Two such examples are abnormalities in Cyclin D and PI3k-RAS binding protein, which helps control the cell cycle. These targets are very important because they can change how tumours grow and spread. To make new molecular treatments and chemotherapy choices, we need to fully understand these molecular targets [2]. Cyclin-dependent kinases (CDK), one of the family of conserved serine/threonine protein kinases, is crucial for maintaining homeostasis and regulating the cell cycle, as well as normal cell proliferation. These traits are replaced by persistent proliferative signaling and growth suppressor evasion during malignant transformation. It is one of the most important oncogenes and has been linked to premalignant lesions and oral cancers. Abundant expression of cyclin D1 has been associated with poor patient outcomes, including aggressive tumors, recurrence, and extended periods of remission. Cyclins, cyclin-dependent kinases (CDKs), and their inhibitors regulate the progression of cells through the different stages of the cell cycle [3]. In numerous cell types, the cyclin D1 proto-oncogene plays a crucial role in controlling the progression of the G1 to S phase. Cyclin D1 forms active complexes with its binding partners, cyclin dependent kinase 4 and 6 (CDK4 and CDK6), which phosphorylate and inactivate the retinoblastoma protein (RB) to promote cell cycle progression [4].

PI3Ks belong to a conserved family of lipid kinases that are divided into three classes based on sequence homology and substrate preference [5]. The only Class I PI3K frequently found to have oncogenic mutations in cancer is PI3Kα. In the PI3K/Akt/mTOR pathway, PI3Kα is the primary Ras effector that phosphorylates

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PIP2 to PIP3. By promoting membrane recruitment and assembling the PIP2 substrate, Ras triggers the activation of PI3Kα. Numerous cellular processes, such as cell division, growth, proliferation, migration, and apoptosis, are mediated by them. PI3K dysfunction is a common feature of human cancer [6]. Oncogenes driven by RAS mutations that affect PI3K (p110) are unable to form or sustain tumors when this RAS binding domain is disrupted. For these reasons, one possible approach for the development of anticancer drugs is to target and inhibit CDK-2 and PI3K- RAS binding protein.

Recent developments in our knowledge of the molecular regulation of these different pathways will enable more precise prognostication and diagnosis, and they may pave the way for more inventive approaches to prevention and treatment. A significant obstacle to the treatment of oral cancer is late diagnosis and the emergence of drug-resistant cancer cells. Since many of the currently prescribed drugs have negative side effects, finding new candidate drugs and targeted therapy is a need that has to be addressed. Medicinal plants have fewer side effects and can contribute to the reduction of resistance to cancer therapy and are valuable sources of drugs due to their bioactive ingredients.

Compared to other structurally related flavonoids, apigenin (APG), a consumable flavonoid (4′,5,7-trihydroxyflavone), has gained popularity as a medication that promotes health because of its low intrinsic toxicity and unique effects on cancer against normal cells [7]. Another plant metabolite, Chysoeriol is a chemically produced substance that comes from the flavone family of chemicals, specifically luteolin [8]. It has intriguing anti-inflammatory, anti-cancer, antioxidative, anti-lipase, anti-xanthin oxidase, and antimicrobial properties,and therefore is of great scientific interest [9]. In Chinese traditional medicine, luteolin is a common natural compound that is used to treat a variety of illnesses, including cancer, hypertension, and inflammatory disorders. Numerous studies have emphasized the various biological effects of luteolin, including its anti-inflammatory, anti-allergy, antidiabetic, neuroprotective, and anticancer qualities. It can also serve as an antioxidant biochemically due to its molecular structure [10].

A crucial tool in computer-assisted drug design and structural molecular biology is molecular docking. Predicting the predominant binding mode(s) of ligands with a protein that has a known three-dimensional structure is the goal of ligand-protein docking. In addition, docking can be used to rank the results, visualize how ligands inhibit the target, and perform virtual screening for larger libraries of compounds or molecules, all of which are useful for lead optimization. Thus the aim of the study is to demonstrate the computer aided drug design to identify a potent natural molecule for targeting cyclinD4 and PI3K RAS binding protein using apigenin, chrysoeriol and luteolin.

Materials and Methods

Ligand selection

The three plant based ligands (Apigenin, Chrysoeriol and Luteolin) were selected and their structures were retrieved from PubChem database in sdf format (Table 1). The 3D structure of ligands was converted into pdb format using openbabel software [11].

Protein retrieval

Three-dimensional structural data of protein receptor molecules was from the public domain in the Protein Data Bank. The structure of the protein targets Cyclin D1 and PI3K-alpha Ras Binding Domain was retrieved from Protein data bank (Table 2). The receptor molecular was processed before docking with ligand such as water molecule and heteroatoms were removed which was relevant to the docking and missing atoms in amino acid residues were deleted and the ligand bound active site in pdb file data was removed in AutoDock Tools [12].

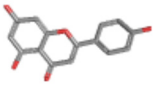
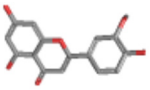
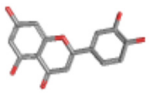
Molecular docking

The process of molecular docking was performed via the Lamarckian Genetic Algorithm by the AutoDock Tools 1.5.7 software [12]. AutoGrid was used to generate a grid box with dimensions of 50 x 64 x 78 Å, with a grid spacing of 0.5 Å. This grid box was specifically centered on the hotspot residues found inside the active site of the target 2W9F and 6VO7. The grid box was positioned at the coordinates (83.35, 49.60, 50.60) in the x, y, and z dimensions, respectively. According to White et al [13], the docking parameters for each molecule were determined based on 100 separate docking experiments, using a maximum of 2.5 x 106 energy assessments, a mutation rate of 0.02, and a crossover rate of 0.8. Docking parameter files (DPF) serve as documentation of the results obtained from the Lamarckian genetic approach. Clustering analysis with a tolerance of 1.0 RMSD was used to analyze the predicted binding poses of each molecule. The representative conformation with the lowest energy from the biggest cluster was chosen. The ligand fragments interactions with the targets, Cyclin D1 and Pi3K RAS binding domain, were observed and analyzed via PyMOL. An easy to use molecular graphics visualization tool which provides structural bioinformatics analysis ‘PyMOL’ is utilized [14].

Results

PyMol was evaluated in order to examine the structure of the proteins. The docking poses were ranked based on the combination of the list of docked ligands, the binding poses that matched those ligands, and the docking scores, respectively. There were ten docking runs that were successfully completed. A distance of 0.375 A was formed between each grid point, and the grid parameters were established in the same manner as was mentioned before. Immediately after the completion of the simulations, the docked structures were investigated, and the interactions were carefully recorded. In order to determine which conformers were the most effective, measurements were taken between the donors and acceptors to determine the binding distance and the hydrogen bond interactions. Upon investigation, it was found that the Van der Waals scaling factor and the Root Mean Square Deviation (RMSD)

Table 1. Structure of Ligands Retrieved from PubChem Database

S.No.	Structure Name	Structure	Structure ID	Molecular Formula
1	Apigenin		PubChem CID: 5280443	C15H10O5
2	Chrysoeriol		PubChem CID: 5280666	C16H12O6
3	Luteolin		PubChem CID: 5280445	C15H10O6

tolerance of different conformation clusters were both equal to 1.0 A.

Interaction with Cyclin D

A single cluster of conformers with an RMSD-tolerance of 1.0̀ was produced by docking simulation of all the ligands into Cyclin-D out of ten docking runs. Table 3 displays the binding energies of the ligands to the protein CyclinD. The binding energies were Luteolin (-5.45 kcal mol-1), Chrysoeriol (-4.99 kcal mol-1) and Apigenin

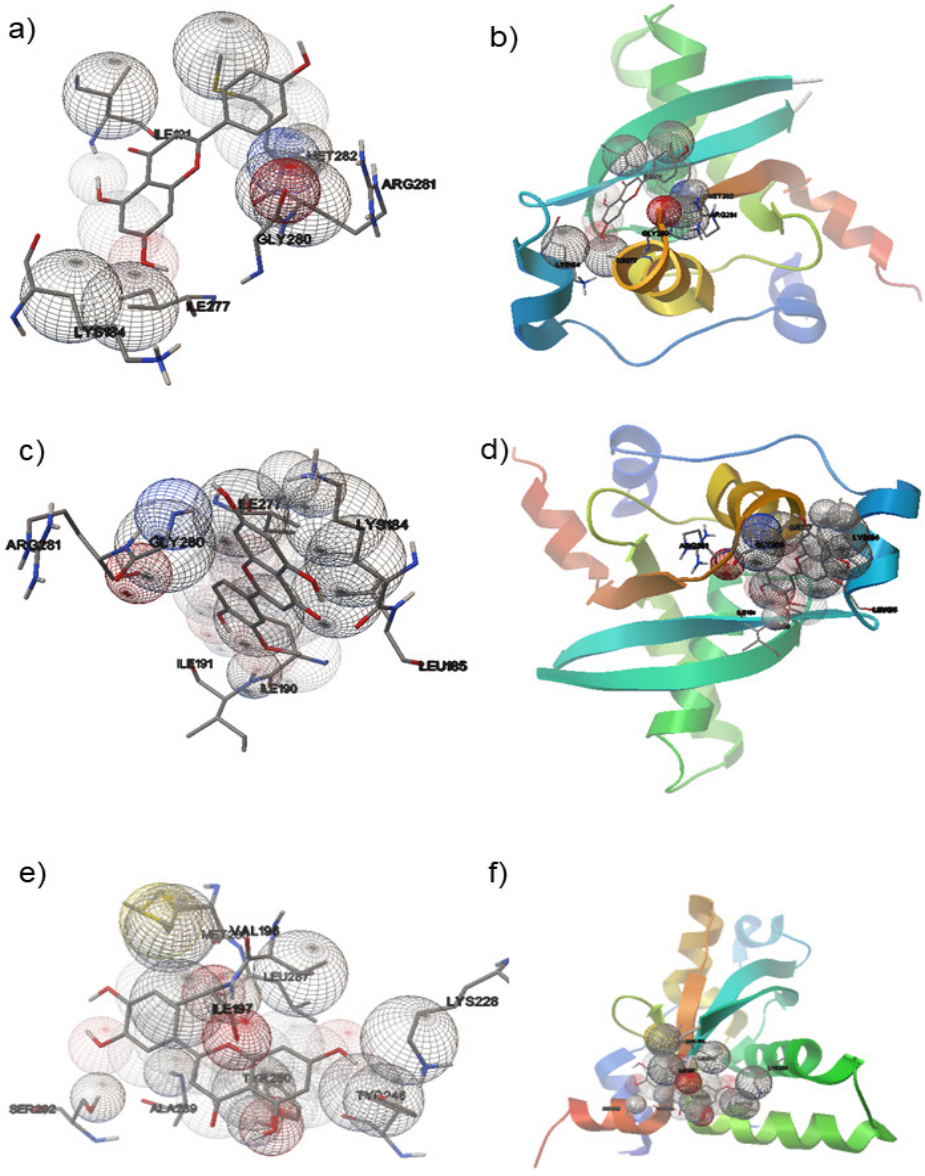
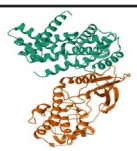
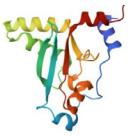


Figure 1. Molecular Docking of Tumor Inducing Cyclin-D Protein Molecule with Plant Based Drugs. a) protein-ligand interaction Cyclin-D with Apigenin, b) Ligand molecule docking against chain B of 2W9F, c) protein-ligand interaction Cyclin-D with Chrysoeriol, d) Ligand molecule docking against chain B of 2W9F, e) protein-ligand interaction Cyclin-D with Luteolin, f) Ligand molecule docking against chain B of 2W9F.

Table 2. Structure of Receptor Molecule Retrieved from Protein Data Bank

S.No.	Structure Name	Structure	Structure ID	No. of Chains and Sequence Length
1	CDK4 in complex with a D-type cyclin		PDB ID: 2W9F	A (271) B (306)
2	PI3K-alpha Ras Binding Domain (RBD)		PDB ID: 6VO7	A (144)

(-4.96 kcal mol-1). The binding energy of luteolin (-5.45 kcal mol-1) was the highest among the three ligands. The residues LEU187; GLU219; ALA220; LEU223; PHE227; PRO239; ARG240; VAL242 are the sites where the hydrogen bond interactions occur.

The present investigation included the molecular docking of the tumor-inducing cyclin-D protein molecule in conjunction with plant-based ligands of apigenin,

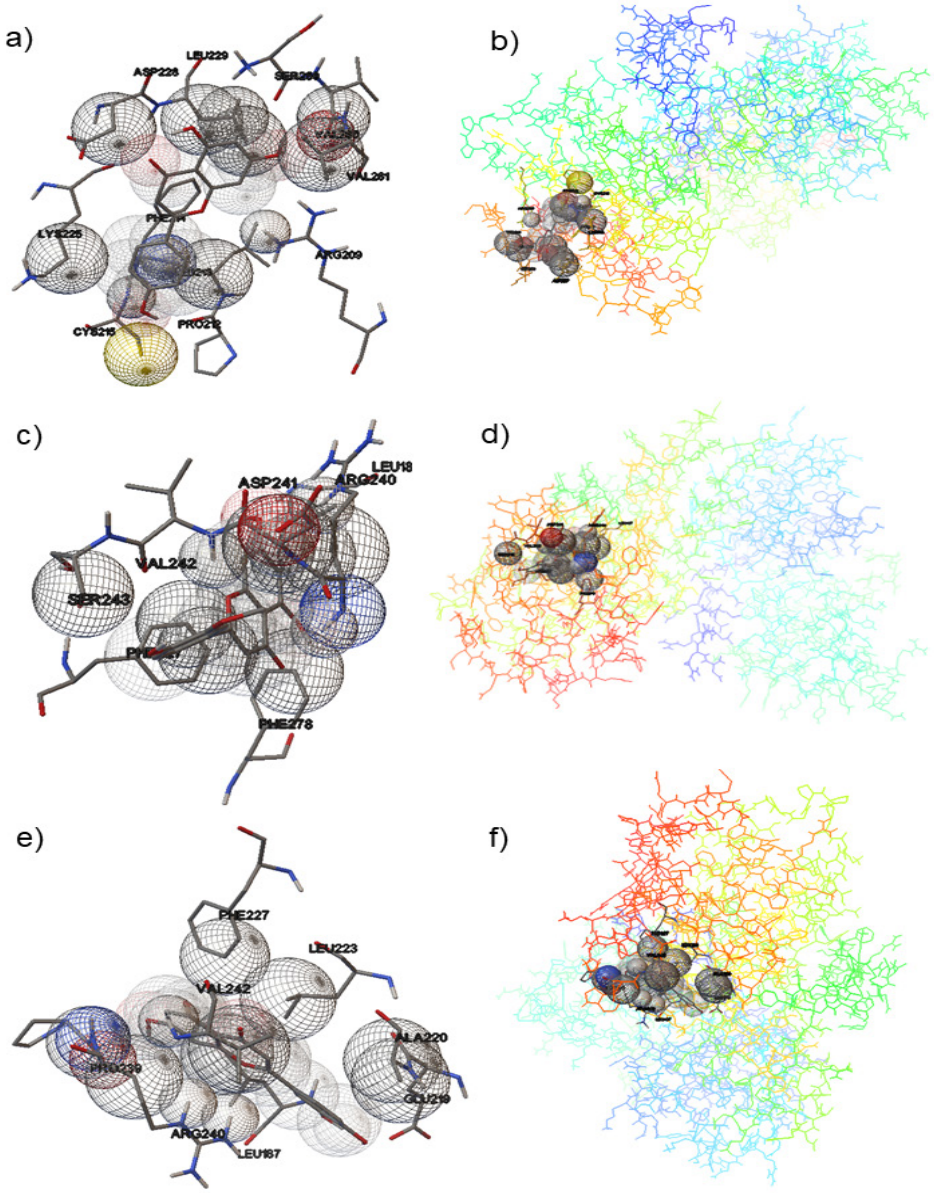


Figure 2. Molecular Docking of Tumor Inducing PI3K-alpha Ras Binding Domain with Plant based Drugs. a) protein-ligand interaction PI3K-alpha Ras Binding Domain with Apigenin, b) Ligand molecule docking against chain B of 6VO7, c) protein-ligand interaction PI3K-alpha Ras Binding Domain with Chrysoeriol, d) Ligand molecule docking against chain B of 6VO7, e) protein-ligand interaction PI3K-alpha Ras Binding Domain with Luteolin, f) Ligand molecule docking against chain B of 6VO7

Table 3. Molecular Docking Result of Cyclin D Protein Receptor Molecule (PDB: 2W9F) with Plant based Drug

RMSD	APIGENIN	CHRYSOERIOL	Luteolin
Binding Energy	-4.96 kcal/mol	-4.99 kcal/mol	-5.45 kcal/mol
Inhibitory Constant	232.03 µM	221.57 µM	100.69 µM
Intermolecular energy	-6.15 kcal/mol	-6.48 kcal/mol	-6.94 kcal/mol
Total Energy	-0.93 kcal/mol	-1.66 kcal/mol	-2.01 kcal/mol
Amino acid residue	Chain B: ARG209; LEU213; PHE214; CYS215; LYS225; ASP228; LEU229	Chain B: LEU187; PHE227; ARG240; ASP241; VAL242; SER243; PHE278	Chain B: LEU187; GLU219; ALA220; LEU223; PHE227; PRO239; ARG240; VAL242

Table 4. Molecular Docking Result of PI3K-alpha Ras Binding Domain Receptor Molecule (PDB: 6VO7) with Plant based Drug

RMSD	APIGENIN	CHRYSOERIOL	Luteolin
Binding Energy	-4.51 kcal/mol	-4.6 kcal/mol	-4.56 kcal/mol
Inhibitory Constant	490.35 µM	421.89 µM	451.0 µM
Intermolecular energy	-5.71 kcal/mol	-6.1 kcal/mol	-6.06 kcal/mol
Total Energy	-0.91 kcal/mol	-1.68 kcal/mol	-2.03 kcal/mol
Amino acid residue	Chain A: LYS184; ILE191; ILE277; GLY280; ARG281; MET282	Chain A: LYS184; LEU185; ILE190; ILE191; ILE277; GLY280; ARG281	Chain A: VAL196; ILE197; LYS228; TYR246; TYR250; MET286; LEU287; ALA289; SER292;

chysoeriol, and luteolin (Figure 1). The ligands' small molecules were subjected to docking with the protein target in order to get insights into the molecular-level interaction. This is of utmost importance in drug design and in comprehending biological function. The ligands were shown using a ball-and-stick model, which was superimposed with a mesh representing the surface of the binding pocket involved in the interaction. The amino acid residues that interacted with the ligand were identified and shown as sticks to illustrate the hydrogen bond interactions. The current findings indicate that luteolin may be the most powerful inhibitor of the Cyclin D receptor molecule among the three substances examined. This discovery might have implications for the development of medicines targeting disorders involving Cyclin D, such as some types of cancer.

Interaction with PI3K-alpha Ras Binding Domain receptor molecule

A single cluster of conformers with an RMSD-tolerance of 1.0̀ was produced by docking simulation of all the ligands into PI3K-alpha Ras Binding Domain receptor molecule out of ten docking runs. Table 4 displays the binding energies of the ligands to the protein PI3K-alpha Ras Binding Domain receptor molecule. The binding energies were Apigenin (-4.51), Chrysoeriol (-4.6) and Luteolin (-4.56). The binding energy of chrysoeriol (-4.6 kcal mol-1) was the highest among the three ligands. The residues LYS184; LEU185; ILE190; ILE191; ILE277; GLY280; ARG281 are the sites where the hydrogen bond interactions occur.

The computational docking of the PI3K-alpha Ras Binding Domain and various plant-based pharmaceuticals (ligands), including apigenin, chrysoeriol, and luteolin, was performed to determine the predicted orientation of a ligand binding to a protein and forming a stable complex (Figure 2). The ligands were attached to the protein receptor, which was shown with a mesh around it to indicate the electron density and the region of molecular surface contact. The stick representations of the amino acids inside the protein illustrate the hydrogen bond interactions that occurred throughout the contacts. These ligands' ability to bind to the protein suggests that they may have inhibitory or modulatory effects. This could be important for the creation of medicines that target the PI3K signaling pathway, which is a system that is often linked to different types of cancer. The structural information obtained from these docking experiments may be very important for the logical development of more powerful and specific medications.

Discussion

One of the most widely used techniques in computer-aided drug design (CADD) to find new therapeutic leads is molecular docking. Molecular docking is a computational method that is generally used to determine the binding orientation and affinity of a molecule to a drug target protein. It is important to characterize the targets in order to comprehend the mechanism of action and the relationship between structure and activity of the therapeutic targets, as well as to direct structural optimization. Discovery and development of natural product drugs will be an integrative strategy that combines the many discovery tools with the recently established field of integrative biology [15]. The molecules selected in our study as ligands are found to possess strong anticancer properties from a biological and pharmacological standpoint, based on previous studies and bibliographical research.

Cyclin D1 is a biomarker of cancer phenotype and disease progression and is frequently dysregulated in cancer, along with the other D-type cyclins to a lesser

extent. The most well-established mechanism for these cyclin's carcinogenic effects is their activation of the cyclin-dependent kinases (CDKs) CDK4 and CDK6, which makes them a desirable target for therapy [16]. From the docking results it can be seen that all the three ligands showed good results in the molecular docking study against the targets while luteolin has the highest binding energy (-5.45) with Cyclin D receptor molecule followed by Chrysoeriol (-4.99) and Apigenin (-4.96). The highest binding Score of luteolin against Cyclin D indicates that luteolin has more stable binding of ligands with the target Cyclin D. This in accordance with the study conducted by Yang et al. [17], wherein luteolin reduced the SCC-4 cells viability and induced apoptosis by reducing the cyclin-dependent kinase (CDKs) expression. This may be because of the ability of Apigenin to initiate the process of apoptosis in cancerous cells and causes cell cycle arrest and decreases the cell viability [18]. Depending on the strength and duration of the oncogenic signal, there is a sequential phosphorylation and activation of RAF-1, mitogen-activated protein kinases (MEKs) 1/2, and extracellular signal-regulated kinases (ERKs) in response to an oncogenic signal. The induction of cyclin D1 transcription is also linked to ERK1/2 activation.

In human cancer, one of the most common causes is aberrant PI3K signaling.Research has demonstrated that in RAS mutant cell lines, PI3K signaling is necessary to sustain transformed growth [19]. Thus, targeted tumor therapy gains momentum with the discovery of particular inhibitors that specifically target this pathway. The binding energy of Chrysoeriol (-4.6) with PI3K-alpha Ras Binding Domain receptor molecule is the highest compared to Apigenin (-4.51) and Luteolin (-4.56). The increased binding affinity of Chrysoeriol against the target is in accordance with the study conducted by Wonlkularb et al. [15], wherein chrysoeriol has been shown to promote apoptosis in rat C6 glioma cells via suppression of the PI3K/Akt/mTOR signaling pathway, thereby demonstrating the potential antineoplastic effects of chrysoeriol on glioma cells. In another study by Wang et al, Chrysoeriol has been found to inhibit the PI3k pathway in pneumonia cells [20]. The results of these studies concurs with the findings of our study that the chrysoeriol has good binding affinity with PI3k-Ras binding protein. Numerous substrates essential in the control of cell survival, cell cycle progression, and cellular growth are modulated by activated Akt. It has been postulated that inhibition in the PI3K-AKT-mTOR pathway decreases the anti-oncogene response, thereby increasing the bypass signal, and eventually the signals in normal cells reach a state of equilibrium [21]. Many of the processes that the novel drugs target involve the PI3K/Akt pathway; hence, a deeper comprehension of this pathway can aid in optimizing the potential advantages of these novel compounds [22,23]. Therefore, the identified drug Chrysoeriol could be used as potential target which inhibits the PI3K/AKT pathway, which could suppress the invasion, proliferation, and migration of oral SCC cells [24]. STAT 3 is a potential therapeutic target in the treatment of melanoma. Chrysoeriol has been shown to inhibit the phosphorylation of STAT3 and caused apoptosis of A375 and B 16F10 melanoma cells [25]. The field of drug discovery has undergone a revolution in the post-genomic era, characterized by an exponential rise in open access biological data generated by bioinformatics pipelines. This has allowed scientists to gain a comprehensive understanding of the biological system relevant to the disease under investigation by utilizing a variety of biological datasets [26-28].

In conclusion, Molecular docking continues to be a promising source for the identification of scaffolds with a wide range of bioactivities and high structural diversity that can be either directly developed or utilized as building blocks for the optimization of new drugs. Based on the molecular docking and binding energy results of this study, Luteolin was identified as a prospective inhibitor against Cyclin D and Chrysoeriol against PI3K-alpha Ras Binding Domain receptor molecule.

Author Contribution Statement

Priyadarshin.G, Carried out the research, wrote the manuscript. Gheena.S, Study conceptualization, Manuscript critical appraisal and correction. E. Dilipan, Carried out the research, Manuscript critical review. Pratibha Ramani, Manuscript critical review.

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Ethical Approval

The study was approved with the SRB number(SRB/SDC/UG-2101/22/OPATH/054) after passing the Ethical committee.

Conflicts of interest

No conflicts of interest

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