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INSILICO EFFICACY OF QUERCETIN AS A THERAPEUTIC POTENTIAL FOR COLORECTAL CANCER

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Abstract:
Multiple lines of evidence show that cancer stem cells inside solid tumors start and maintain tumor growth. These cells stay dormant even after anti-cancer drugs are used to treat the tumor. Different flavonoids might help protect against cancer by messing up pathways that are linked to cancer stem cells. Flavonoids like quercetin are used in many medicine ways. By acting as an antioxidant and an anti-inflammatory, quercetin can stop the growth of cancer stem cells. It can also control many molecular processes that are linked to the growth of cancer. As a chemical plan, it can be used in combinatorial synthesis. It is possible to find molecular targets. Figuring out exactly which molecules quercetin targets could help scientists make more precise molecules that only go after those shared spots. Synergistic effect: Now that we know more about how quercetin works at the molecular level, we can look into how it might work with other substances to help avoid cancer. The goal of this study was to make nanoparticles that contain quercetin so that they can be used in specific drug delivery systems. Then, several cancer stem cell types were used to test these nanoparticles.

Keywords: Molecular docking, Antioxidant, Cancer-stem cells, Quercetin, Flavonoid, Nanoparticle, Synergistic effect.

Introduction:

Cancers are a collection of diseases characterized by abnormal cellular growth. These cells have the ability to invade and metastasize to other regions of the body (Saleem, *et al.,* 2022). While malignant tumors proliferate and metastasize to other regions of the body, benign tumors remain localized (Eseyin, *et al.,* 2022). There exist over 200 distinct forms of cancer (Wadanambi, *et al.,* 2023). Most of them are caused by chemicals or other harmful substances, nuclear radiation, infections, or genes (Surana, *et al.,* 2021). Lifestyle choices, like what you eat and how exposed you are to the surroundings, can have a big effect (Surana, *et al.,* 2021). To avoid many types of cancer, you should not smoke, keep your weight in a healthy range, drink alcohol in moderation, eat lots of fruits, vegetables, and whole grains, get vaccinated against some infectious diseases, eat less processed and red meat, and spend as little time in the sun as possible (Pandya, *et al.,* 2020). Cancers like cervical and bowel can be found early with the help of screening (Pathan, *et al.,* 2023). The pros and cons of breast cancer screening are still being talked about. One important part of care is dealing with pain and symptoms in a good way (Surana, *et al.,* 2021). Help with pain and other symptoms are very important for people who are very sick. How well someone will do with treatment depends on what kind of cancer they have and how far along it is (Mondal, *et al.,* 2022). In developed countries, 80% of children under 15 who are identified usually live for five years after being told they have cancer. In the US, 66% of people with cancer are still alive after five years (Kaloni, *et al.,* 2020). Colon cancer (CRC) is the most common cancer in the world and kills and sickens many people every year (Iman, *et al.,* 2016). Colorectal cancer (CRC) could happen to both men and women, but men were more likely to get it than women. Additionally, an amazing 90% of people who are diagnosed with colon cancer are 50 years or older, and the chance of getting this disease rises with age (Ahire, *et al.,* 2020).

When genetic and epigenetic changes slowly build up in the healthy lining of the gut, they cause invasive cancer to grow (Gaikwad, *et al.,* 2023). This is called colorectal cancer. It has been found that colorectal cancer has molecular paths that aren't working right, which shows how diverse it is (Keservani, *et al.,* 2020). The chromosomal instability (CIN) route is what causes chromosomal abnormalities and loss of heterozygosity (LOH). It is thought to be the cause of 70–85% of colorectal cancer cases (Surana and Mahajan, 2022). Colorectal cancer (CRC) is often linked to microsatellite instability (MSI), which happens when the DNA Mismatch Repair (MMR) system doesn't work right. This factor is responsible for about 15% of random CRC cases (Ghasemzadeh, *et al.,* 2014). Colorectal cancer usually spreads in three main ways. The third is through the CpG island methylator phenotype (CIMP) route (Maheswari and Sankar, 2024). This condition is caused by the abnormal overmethylation of CpG dinucleotide sequences found in the promoter regions of genes that control cell cycle, angiogenesis, invasion, and binding (Ahmadipour, *et al.,* 2015). The result is that gene production stops. Due to the seriousness of colon cancer, it is important to find and create new ways to prevent and treat it right away (Kamalidehghan, *et al.,* 2018). A computer-based docking study using different random proteins explains why quercetin can bind to different things in different ways (Mohan, *et al.,* 2013).

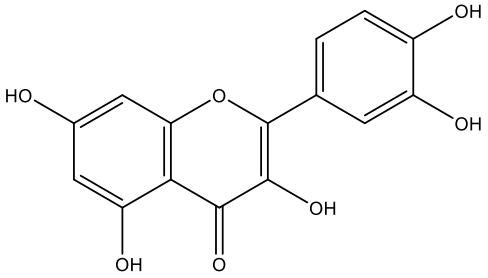


Figure 1: Structure of Quercetin

Materials and Methods:

Preparation Ligand for docking:

The ligand Quercetin's 3D shape was found in the PubChem Open Chemistry Database, and AutoDock Tools were used to make it even better (Iman, *et al.*, 2015).

Proteins Preparation for docking:

The target proteins' PDB file was downloaded from the RCSB Protein Data Bank. The correct PDB ID can be found in Table 1. Using PyMol, the starting protein structure was changed into a single-molecule receptor. It was then changed even more by adding polar hydrogens. The Auto Dock Tools (ADT) program was used to figure out the gasteiger charges and get rid of any ligand torsional bonds (Parkhe, *et al.*, 2023 and Sharma, *et al.*, 2018).

Molecular Docking Studies for Quercetin with Proteins:

The AutoDockVina tool was used to do molecular docking simulations to look into how quercetin and proteins associate with each other. Table 1 shows the grid sizes and centers of XYZ points, with 1 unit between each grid. The docking parameter file was used to prepare the text page. The Lamarckian genetic method was used to guess how molecules would join together, and the binding energies were used to check the results. For everything else, the default settings were used (Keservani, *et al.*, 2019 and Sekar, *et al.*, 2021).

Table 1: Grid mapping of selected target proteins for molecular docking

Sr. No.	**Protein Receptors**	**PDB ID**	**Grid Size**			**Grid Center**		
			X	**Y**	**Z**	**X**	**Y**	**Z**
1	Protein Kinase B	1UNR	46	40	155	12.356	-2.332	-0.006
2	CDK8	3RGF	83	77	83	-2.890	4.499	12.674
3	BUBR1	3SI5	119	106	83	30.683	5.024	22.005
4	APC	3NMZ	74	80	76	39.464	-35.473	-15.899
5	CK2α	3WAR	83	68	81	-9.187	-5.094	9.013
6	FABP6	5L8I	88	56	82	50.866	-3.069	79.121
7	KRas	4OBE	61	64	94	-8.687	-25.161	29.411
8	Bcl-xL	1MAZ	48	45	60	2.495	23.599	41.558
9	Bcl-2	2XA0	93	76	88	53.738	17.134	-20.970

RESULTS AND DISCUSSION

Molecular docking:

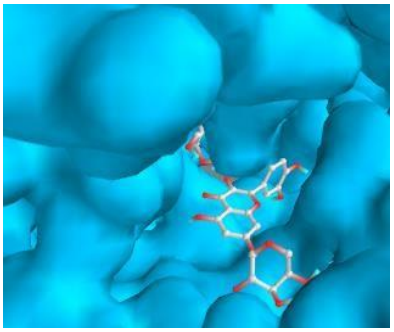
Employing molecular docking to determine the interaction between Quercetin and proteins is a distinctive approach. This study focused on determining the binding energy scoring function of quercetin with various types of inflammatory and anti-apoptotic proteins. Additionally, we conducted a computational analysis to understand how quercetin interacts with these proteins in colorectal cancer (CRC). Table 2 presents a summary of the docking scores between quercetin and proteins associated with inflammation and anti-apoptosis.

Table 2: Binding energy of molecular docking study

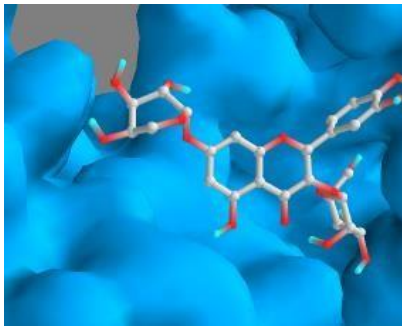
Sr. No.	**Receptors for molecular docking**	**PDB ID of Protein**	**Docking score (kcal/mol)**
1	Protein Kinase B	1UNR	-10.3
2	CDK8	3RGF	-8.6
3	BUBR1	3SI5	-8.2
4	APC	3NMZ	-9.4
5	CK2α	3WAR	-8.9

6	FABP6	5L8I	-6.7
7	KRas	4OBE	-8.9
8	Bcl-xL	1MAZ	-8.6
9	Bcl-2	2XA0	-9.3

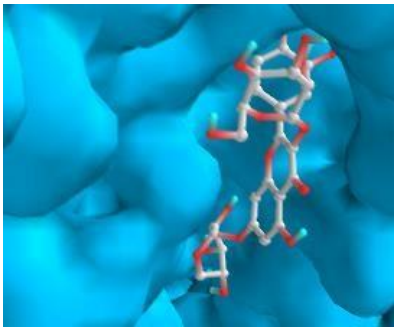
The efficacy of Quercetin-protein complex formation is evidenced by the negative binding energy observed between Quercetin and protein. Quercetin, with a binding energy of -10.3, has the highest stability when forming a complex with protein kinase (PDB ID: IUNR). Quercetin establishes hydrogen bonds and other non-interactive interactions within the active pockets of the proteins discussed before (Figure 1). All the proteins tested in this experiment exhibited strong binding energies, ranging from -10.3 to -6.7, thereby verifying the binding.



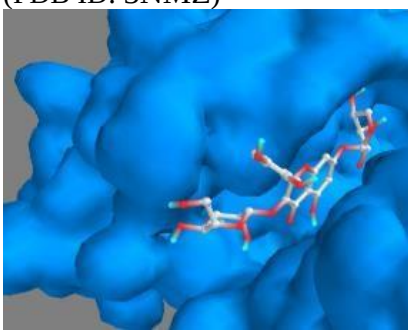
(A): **Protein Receptors**: APC (PDB ID: 3NMZ)



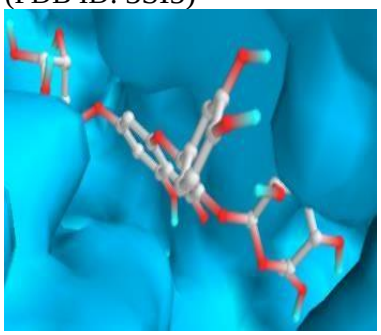
(B) **Protein Receptors**: BUBR1 (PDB ID: 3SI5)



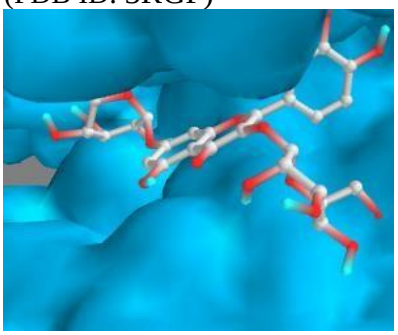
(C) **Protein Receptors**: CDK8 (PDB ID: 3RGF)



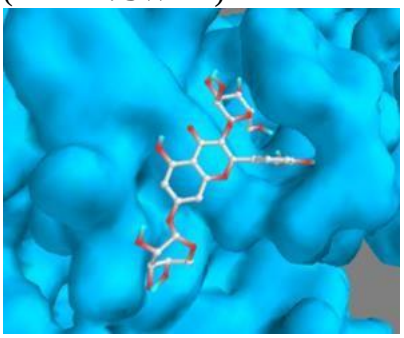
(D) **Protein Receptors**: CK2a (PDB ID: 3WAR)



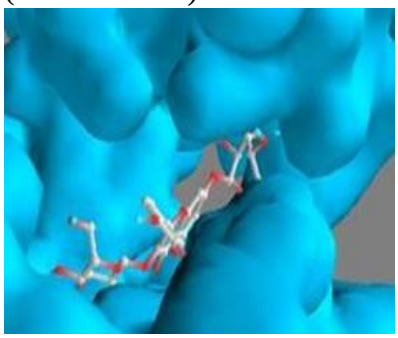
(E) **Protein Receptors**: FABP6 (PDB ID: 5L8I)



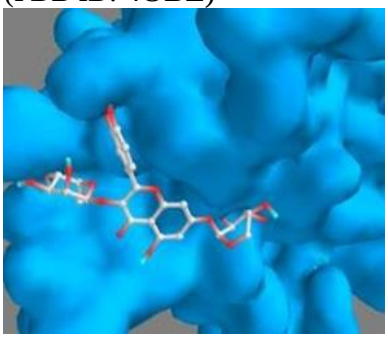
(F) **Protein Receptors**: KRas (PDB ID: 4OBE)



(G) **Protein Receptors**: Bcl-xL (PDB ID: 1MAZ)



(H) **Protein Receptors**: Bcl-2 (PDB ID: 2XA0)



(I) **Protein Receptors**: Protein Kinase B (PDB ID: 1UNR)

Figure 2: Ligand Interaction diagram of Quercetin with respective protein

The adenomatous polyposis coli (APC) gene is responsible for the activation of the CIN pathway, which leads to increased expression of the Wnt/β catenin pathway. This pathway is

accountable for the formation of 70–85% of colorectal malignancies. The main cause of this is the mutation in the APC gene. The APC protein is essential for regulating the degradation of cytoplasmic β catenin. Hence, in the case of a mutation in the APC protein, there is an accumulation of cytoplasmic b-catenin which binds to the Tcf transcription factor family. This alteration in turn affects the expression of several genes that influence apoptosis, migration, proliferation, and differentiation, ultimately leading to the development of colorectal cancer (CRC). Our in silico investigation revealed that quercetin forms strong bonds with several amino acid residues of the APC protein, namely HIS672(2), ASN627(2), TYR175(2), THR628, TRP242(2), LEU629(2), GLU633, and SER172, resulting in a highly favorable binding energy of -9.4 Kcal/mol.

CDK, also known as cyclin-dependent kinase CDK8: CDK8 governs the mechanism by which B-catenin stimulates gene transcription within the nucleus, hence overseeing the transcriptional regulation of the Wnt/β catenin pathway. A considerable proportion of colon malignancies exhibit a response in the CDK 8 gene, and a study conducted by Firestein et al. (2008) demonstrated that inhibiting CDK8 expression halted the proliferation of colon cancer cells. Our research revealed that quercetin establishes a long-lasting interaction with specific amino acid residues GLN161, TRP6(2), TYR162, ARG157(3), PHE195(3) PRO158(2), during the docking process with CDK8.

KRAS, a protooncogene, is frequently mutated (30–60%) in colorectal cancer. The main mutation is a genetic alteration that enhances the activity of the Wnt/β catenin pathway, leading to an increase in signaling. This, in turn, results in the growth of polyps in the colon. The activation of KRAS is commonly triggered by the attachment of GTP, and extensive research is being conducted to inhibit the generation of active KRAS-GTP.

Akt kinase, often referred to as protein kinase B, plays a crucial role in transmitting signals from oncogenes and growth factors to downstream targets that control critical elements of tumor formation. The AKT pathway is commonly hyperactivated in colon cancers, making it one of the most frequently observed signaling pathways in this kind of cancer. Thus, it has been suggested that inhibitors targeting the PI3K/Akt signaling pathway could serve as valuable therapeutic drugs. Our research findings indicate that quercetin forms a stable interaction with Protein kinase B at an energy level of -10.3 Kcal/mol. This interaction has the potential to inhibit the hyperactivation of the Akt signaling pathway by preventing the phosphorylation of Akt kinase.

Bcl-xL and Bcl-cell lymphoma There are two types of proteins known as Bcl-2 proteins. These proteins, referred to as anti-apoptotic proteins, play a crucial role in regulating the pathway that leads to mitochondrial cell death. In typical physiological conditions, they inhibit the activity of pro-apoptotic proteins like as Bak and BAX. The expression of these proteins is increased in cancer cells, leading to the disruption of the apoptotic process. The experiment showed that inhibiting these proteins can increase the number of cancer cells that undergo apoptosis and reduce the amount of tumor growth. Our docking investigations indicate that quercetin forms interactions with the amino acids of Bcl-2 and Bcl-xL, resulting in binding energies of -8.6 and -9.3 Kcal/mol, respectively. This indicates that quercetin has the potential to restrict the activity of anti-apoptotic proteins, hence promoting the apoptosis process.

BUBR1 is a crucial constituent of the mitotic checkpoint complex. Its main function is to delay the onset of anaphase until all chromosomes have properly connected to the spindles. In this way, BUBR1 oversees the mitotic spindle checkpoint. Genetic variations in BUB1B, a gene responsible for encoding BUBR1 and MAD2 proteins, result in heterozygous mutations. These mutations promote premature separation of chromatids, which might potentially contribute to the onset of cancer. It was found that the expression of BUBR1 has been altered

in CRC cell lines. Thus, these proteins can be considered as promising targets for the development of advanced therapeutic regimens for CRC. Considering this, we performed a docking of quercetin with the BURB1 protein and obtained a favorable binding score of -8.2 Kcal/mol.

CK2α is a protein serine/threonine kinase that is broadly distributed and highly conserved. It plays a crucial role in regulating several stages of the cell cycle, most likely via phosphorylating proteins that are involved in promoting cell cycle progression. Zouet et al. (2011) found that colorectal cancer cells have higher levels of CK2α expression, and the proliferation of CRC cells is reduced by inhibition of this protein kinase. Our analysis revealed a stable link between quercetin and CK2α, indicating that quercetin has the capacity to reduce the activity of CK2α protein kinase.

Elevated amounts of bile acids lead to oxidative damage, inflammation, and excessive growth of colorectal cancer (CRC). FABP6 serves as the primary transporter of bile acids to the epithelial cells in the ileum. Through experimentation, it was shown that this protein is excessively produced, and that suppressing this transporter could reduce the rate at which the tumor grows. Based on this assumption, we conducted a docking experiment between quercetin and FABP6. The results showed a favorable binding score of -6.7 Kcal/mol, indicating that quercetin has the potential to act as an inhibitor of bile acid transport to the colon mucosa. This inhibition could potentially restrict the progression of colorectal cancer.

Conclusion:

Successful molecular docking of Quercetin with many target proteins, such as APC, BUBR1, CDK8, CK2α, FABP6, KRas, BCL–XL, BCL2, Protein Kinase B, and CK2α, was achieved using Auto dock Vina. APC, K-ras, and CDK8 are three of the 14 proteins that have been successfully docked and are believed to have a role in the Wnt/β catenin system. The docking analysis suggests that quercetin has the potential to effectively block the hyperactivated Wnt/β catenin pathway. Upon docking quercetin with the proteins Bcl-2 and BclxL, it was observed that identical results were obtained. These proteins play a role in the process of programmed cell death known as apoptosis. Based on the docking finding, quercetin possesses the capacity to modulate the activation of this enzyme, specifically in relation to the colon's processing of food and drugs. The docking of quercetin and FABP6 protein indicated that quercetin has the potential to regulate FABP6 activity, hence controlling the transport of bile acids. The enzyme CK2α is highly expressed and acts as a link between many pathways, such as the Akt and Wnt/β catenin pathways. Based on the docking result, it appears that quercetin has the potential to regulate CK2α activation, hence restricting the signal transduction linked to these pathways. An in silico examination of the molecule with target proteins suggests that Quercetin shows promise as a potential drug for the treatment of colorectal cancer and other types of cancer as well. Further in vivo and in vitro studies are necessary to evaluate the properties and potential of quercetin as an anticancer drug.

Declarations:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

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