INSILICO DOCKING, PHARMACOKINETIC AND TOXICITY STUDIES OF SELECTED PHYTOCONSTITUENTS: TARGETING COLON CANCER

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Abstract

Colorectal cancer is one of the most frequent diseases in the world and is also one of the leading causes of cancer death. Chemotherapy medications are often limited owing to different consequences, including resistance and recurrence. The *in-silico* docking study included protein or nucleotide exploration, 3D structure modeling, molecular docking, and binding energy estimation. Protein-protein interactions are important in many biological processes, and disruption of these interactions is a main cause of illness. Small molecules are increasingly being used to control proteins, yet protein interfaces often lack holes for processing small molecules. In the molecular docking investigation of 5-fluorouracil, Curcumin, Vanillic acid, Hesperidin, Resveratrol, Fisetin, and EGCG (Epigallocatechin gallate) are the drugs to interact with proteins 1C5Y, 1N8Z, 3BBF, 1UEA, 3BBB, and 6VTC were employed. According to molecular docking and ADMET data, the compounds Fisetin, EGCG, and Hesperidin exhibited the greatest binding energy scores with the majority of the target proteins. The findings imply that it might be utilized to design novel cancer therapies.

Keywords Colorectal cancer, molecular docking, Binding score, Anti-apoptotic proteins, ADMET.

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Introduction

Colon cancer is one of the most frequent malignancies in the world, accounting for about 70% of all cancer patients. Colorectal cancer (CRC) is the third most common cancer type globally and the second greatest cause of mortality in the United States.[1] Colorectal cancer (CRC) is the third most common malignancy in both sexes and the second leading cause of death. Cancer accounts for 10% and 9.2% of all cancer cases in men and women, respectively, and kills over 500,000 people each year. In the United States, about 147,950 new cases of CRC are projected in 2020, with an estimated 53,200 deaths. In Germany, one in every 14 men and one in every 18 women will be diagnosed with CRC throughout their lifetime, and one in every 32 men and one in every 39 women will die from CRC. Colon cancer may be treated easily in the early stages, but after it has progressed to the final stage, it is tough to cure and hurts the patient's health and a slew of symptoms.[2] However, this article will discuss how to cure colon cancer using natural pharmaceuticals with no side effects. It will also aid in the entire treatment of colon cancer. Herbal medicine is the source of almost all current therapeutic drugs, as they are either unchanged dietary supplements or improved synthetic counterparts. Researchers are interested in screening herbal plants, isolating, identifying, and analyzing their secondary metabolites as possible therapeutic leads for the same reason.[3] Some flavonoids, triterpenoids, and polyphenols present in nature have been shown to cause endoplasmic reticulum stress-induced tissue damage via apoptosis and necrosis. [4] Bioactive substances have been changed to increase therapeutic efficacy, bioavailability, specificity, and several other properties, including the incorporation of certain prospective chemotherapeutic drugs. However, their exorbitant cost, adverse effects, medication interactions, and drug resistance difficulties spurred researchers to seek fess priced and more effective alternatives. The identification of molecular targets related to Colon cancer metabolism is currently required for the rational design of effective anticancer medicines.[5]

A huge investment of time, people, resources, and money is being made in psychological tactics to objectively analyze novel biological targets and drugs that efficiently target them. Once the medicine under inquiry has been identified, tests are carried out to see whether the medication has the intended effect on cell culture and experimental animals. Following that, a sufficient number of people should be recruited for clinical research, the majority of side effects should be studied, and specific standards should be followed. During the clinical trials phase, budgets increase.[6] As a result, *in-silico* pre-screening interactions were required to select probable candidates for lab evaluation. This pre-experiment *in-silico* technique greatly minimizes the cost of the experiment. One of every 5,000 drugs ends up in a pharmacy, meaning that pharmaceutical companies spend a massive amount of money. Finding shortcuts and pathways from basic science to clinical research through translational research will thus be extremely beneficial to this field of study. The binding affinity of ligand molecules is computed via molecular docking, which is significant in understanding their metabolic activities. Typically, finding the protein target and its regulator is the initial step in looking for new pharmacological drugs.[7] Protein-protein interactions are important in many biological processes, and disruption of these interactions is a main cause of illness. Small molecules are increasingly being used to control proteins, yet protein interfaces often lack particular holes for processing small molecules. Furthermore, because protein-ligand interactions are critical in drug design, the current study employed molecular docking. The major goal was to find proteins that may be used as chemotherapeutic agents in colorectal cancer utilizing molecular docking and perform thorough research to guarantee that they were correct and could contribute to novel treatment options. **5-fluorouracil** is used as a standard drug which is commonly used for the treatment of CRC.[8] **Curcumin** is a brilliant yellow substance generated by *Curcuma longa* plants. It is the main curcuminoid found in turmeric (*Curcuma longa*), a member of the *Zingiberaceae* ginger family.[9] **Vanillic acid** (44-hydroxy-3-methoxy benzoic acid) is a flavoring ingredient derived from dihydroxybenzoic acid. It's an oxidized version of vanillin. It is also used as an intermediary in the synthesis of vanillin from ferulic acid.[10] **Hesperidin -** Citrus fruits include the flavanone glycoside hesperidin. Hesperetin is the aglycone form. Its name is taken from the word "hesperidium," which refers to citrus tree fruit.[11] **Resveratrol** is a stilbenoid, a kind of natural phenol, and a phytoalexin generated by numerous plants in reaction to damage or when pathogens such as bacteria or fungus attack the plant. Resveratrol may be found in the skin of grapes, blueberries, raspberries, mulberries, and peanuts.[12] **Fisetin** is a plant flavonol in the polyphenol flavonoid category. It is present in many plants and acts as a yellow/ochre coloring pigment. Many

fruits and vegetables contain it, including strawberries, apples, persimmons, onions, and cucumbers.[13] **EGCG** (Epigallocatechin gallate), also known as epigallocatechin-3-gallate, is a kind of catechin that is an ester of epigallocatechin and gallic acid. The most prevalent catechin in tea, EGCG, is a polyphenol being studied for its potential to alter human health and illness.[14] The results of this investigation show a substantial correlation between the PDB structures of genes such as 1C5Y, 1N8Z, 3BBF, 1UEA, 3BBB, and 6VTC.[15] As pharmacological techniques, the majority of these cancer-related genes might be utilized to create anticancer medicines. The research of this link gets yields encouraging results on a potential new colorectal cancer treatment that has to be evaluated in the laboratory.[16]

Methods

Obtaining the protein structure

The 3-D crystal structures of six distinct proteins, 1C5Y, 1N8Z, 3BBF, 1UEA, 3BBB, and 6VTC, were acquired from the Protein database and are largely related to colorectal cancer etiology. A protein data bank is a biological substance library that holds three-dimensional structural information. The receptors' active or binding sites were found utilizing internet servers. They are a visual dataset that depicts the numerous molecules that make up the structure as well as the schematics of its linkages. The protein structure's protein database ID was typed into the homepage's search box.[17]

Protein and ligand structure preparation

The complicated protein structures were created using the protein preparation wizard, the Schrödinger program. Automatically, hydrogen ions were introduced, and the structure was improved and reduced. The structures of 5-fluorouracil, Curcumin, Vanillic acid, Hesperidin, Resveratrol, Fisetin, and EGCG (Epigallocatechin gallate) were obtained from the PubChem database. Using LigPrep, a Schrödinger module, we prepared all of the ligands for docking studies. Tautomers were created a optimizes for each ligand.[18]

3D structure of the proteins and binding site residues

The RCSB PDB was used to obtain the 3D structures of nine distinct proteins implicated in colorectal cancer. Figure 1 to 6 shows the PDB ID, amino acid sequence length, chain chosen for docking investigation, and binding site residues received from internet services.[19,20]

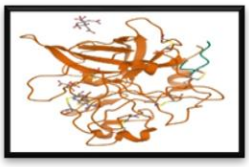

Figure 1: 1C5Y

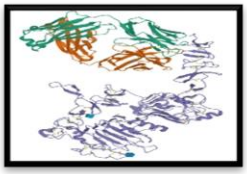

Figure 2: 1N8Z

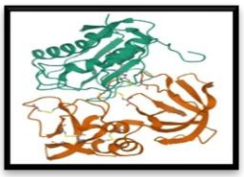

Figure 3: 1UEA

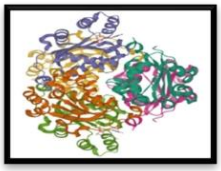

Figure 4: 3BBB

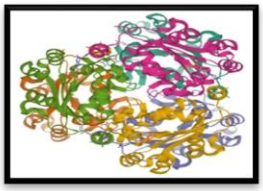

Figure 5: 3BBF

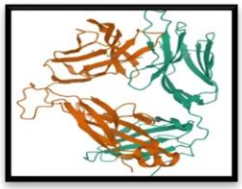

Figure 6: 6VTC

In-silico validation

In-silico docking studies were performed using Schrödinger software and the pharmacokinetic and toxicity studies were performed using ADMETlab 2.0 online tool and the results are mentioned in table 1-10.

Results

Table 1: Insilico Docking score of the selected phytoconstituents

PH PR	5 - Fluorouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
1C5Y	-3.84905	-7.57667	-5.11684	-6.17967	-7.1367	-8.89323	-4.68654
1N8Z	-3.90861	-7.5512	-6.81191	-6.4752	-10.7456	-7.30436	-8.43157
1UEA	-5.61598	-8.33394	-6.97804	-5.9762	-11.2734	-7.46347	-7.43579
3BBB	-3.00519	-6.66753	-4.08292	-5.02712	-8.54309	-6.85968	-7.76339
3BBF	-5.6411	-7.17509	-4.92301	-4.94636	-9.46347	-7.51816	-7.27607
6VTC	-2.21102	-5.68902	-4.69428	-4.80373	-9.302	-6.29451	-9.42211

PH: Phytoconstituents; PR: Protein

Table 2: Structural properties of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
Molar Weight	132.01	368.13	168.04	228.08	610.19	286.05	458.08
Volume	106.916	381.036	162.983	241.503	563.46	273.977	425.17
Density	1.235	0.966	1.031	0.944	1.083	1.044	1.077
logS	-1.279	-3.921	-1.771	-2.273	-3.791	-3.704	-3.917
logP	-0.773	2.742	1.396	2.994	0.642	2.428	1.893
logD	-0.283	2.82	3.428	3.363	0.016	2.043	0.652
Stereo Centers	0	0	0	0	11	0	2
TPSA	65.72	93.06	66.76	60.69	234.29	111.13	197.37

Table 3: Medicinal chemistry of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
QED	0.485	0.548	0.693	0.692	0.185	0.511	0.212
SA score	2.659	2.426	1.542	2.112	4.818	2.359	3.74
Fsp3	0.0	0.143	0.125	0.0	0.536	0.0	0.136
MCE - 18	6.0	14.0	7.0	11.0	120.977	18.0	87.48
Lipinski	Accept	Accept	Accept	Accept	Reject	Accept	Reject
Pfizer rule	Accept	Accept	Accept	Accept	Accept	Accept	Accept
GSK rule	Accept	Accept	Accept	Accept	Reject	Accept	Reject
NP score	-0.799	0.722	0.524	0.754	1.945	1.335	1.65

Table 4: Absorption of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
Caco-2 Permeability	-4.998	-4.834	-5.159	-4.916	-6.693	-4.987	-6.717
MDCK Permeability	1.3e-05	1.6e-05	9e-06	1.4e-05	4.3e-05	1e-05	5e-06
Pgp-inhibitor	0.0	0.284	0.001	0.455	0.001	0.005	0.022
Pgp-substrate	0.011	0.014	0.003	0.102	0.999	0.008	0.001
HIA	0.988	0.06	0.013	0.012	0.819	0.009	0.809
F 20%	0.003	0.011	0.01	0.264	0.001	0.923	1.0
F 30%	0.995	0.171	0.655	0.055	0.998	0.994	1.0

Table 5: Distribution of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
PPB	13.01%	99.79%	53.16%	97.26%	74.50%	97.04%	88.24%
VD	0.39	0.369	0.358	0.822	0.513	0.477	0.514
BBB Penetration	0.051	0.103	0.439	0.032	0.117	0.009	0.006
Fu	77.96%	1.049%	36.01%	2.620%	19.63%	5.170%	8.135%

Table 6: Metabolism of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
CYP1A2 inhibitor	0.031	0.593	0.042	0.976	0.028	0.95	0.345
CYP1A2 substrate	0.978	0.758	0.69	0.11	0.083	0.114	0.071
CYP2C19 inhibitor	0.051	0.287	0.038	0.229	0.047	0.097	0.025
CYP2C19 substrate	0.054	0.12	0.052	0.056	0.163	0.046	0.034
CYP2C9 inhibitor	0.01	0.661	0.052	0.356	0.018	0.535	0.594
CYP2C9 substrate	0.129	0.906	0.16	0.957	0.26	0.689	0.18
CYP2D6 inhibitor	0.006	0.037	0.015	0.629	0.102	0.532	0.012
CYP2D6 substrate	0.038	0.895	0.13	0.915	0.181	0.329	0.171
CYP3A4 inhibitor	0.006	0.674	0.039	0.943	0.049	0.62	0.097
CYP3A4 substrate	0.191	0.517	0.07	0.163	0.039	0.098	0.089

Table 7: Excretion of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
CL	9.342	13.839	7.899	15.661	1.701	8.273	14.45
T1/2	0.938	0.948	0.941	0.924	0.391	0.92	0.933

Table 8: Toxicity of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
hERG Blockers	0.021	0.214	0.031	0.109	0.122	0.043	0.141
H-HT	0.867	0.475	0.224	0.374	0.239	0.127	0.365
DILI	0.973	0.895	0.857	0.032	0.96	0.978	0.941
AMES Toxicity	0.067	0.234	0.015	0.076	0.569	0.73	0.261
Rat Oral Acute Toxicity	0.936	0.896	0.053	0.451	0.11	0.178	0.042
Skin Sensitization	0.22	0.958	0.154	0.959	0.915	0.919	0.969
Carcinogen city	0.081	0.706	0.062	0.287	0.599	0.147	0.034
Eye Corrosion	0.005	0.007	0.202	0.047	0.003	0.004	0.003
Eye Irritation	0.891	0.792	0.988	0.97	0.181	0.93	0.936
Respiratory Toxicity	0.07	0.951	0.12	0.405	0.689	0.074	0.021

Table 9: Environmental toxicity of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
Bioconcentration Factors	0.266	0.8	0.402	0.885	1.113	1.056	0.989
IGC50	1.672	5.077	2.662	3.993	5.036	4.714	4.201
LC50FM	2.63	6.191	2.88	4.334	7.224	5.305	5.256
LC50DM	4.639	5.194	3.56	4.883	7.122	5.333	5.673

Table 10: Toxicophore Rules of the phytoconstituents

	5 - Flurouracil	Curcumin	Vanillic acid	Resveratrol	Hesperidin	Fisetin	EGCG
Acute Toxicity Rule	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts
Genotoxic Carcinogenicity Rule	0 alerts	1 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts
Non Genotoxic Carcinogenicity Rule	0 alerts	1 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts
Skin Sensitization Rule	0 alerts	8 alerts	4 alerts	5 alerts	8 alerts	5 alerts	9 alerts
Aquatic Toxicity Rule	1 alerts	2 alerts	0 alerts	0 alerts	2 alerts	0 alerts	0 alerts
Non-Biodegradable Rule	1 alerts	1 alerts	0 alerts	1 alerts	1 alerts	1 alerts	1 alerts
Sure ChEMBL Rule	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	1 alerts

Discussion:

Here, we learned about docking results for the CRC by interaction with the selected protein or ligand. In this study, we used a variety of different techniques, including pharmacophore modelling, virtual screening, and molecular modelling, to create a cost-effective, effective, and side-effect-free drug. These results can be confirmed based on the docking score so that it will be easy for our future studies to make a formulation of the product. It helps us to save huge money and manpower from clinical trials and pre-clinical studies.

Treatment for CRC is based on these results.

- Helps to make tablets based on the results of the CRC.
- We can make in-situ gel formation in the oral route method.
- And we can make in-situ gel formation based on the injection techniques.
- In-situ gel formation based on pH or temperature degradation techniques.
- It gives ideas to make suppositories in the Rectal route of administration.
- Solid Lipid Nanoparticles (SLN) can be formulated for drugs with less solubility and less permeability or both.
- SLN formulation for making tablets, and injections. In-situ gel, suppositories.

These are the methods of possible ways to treat CRC without doing surgical dissection or Chemotherapy method.

Conclusion:

Nowadays it's easy to treat Colorectal Cancer (CRC) at the early stage for physicians, but when it reaches the last stage, CRC won't be easy to treat or cure patients without surgical dissection or Chemotherapy methods. When patients were going with these methods its caused side effects to them. Based on synthetic medicine which gives severe side effects to them, Chemotherapy causes severe pain to the patients. On the other hand side, natural or phytoconstituents can cure cancer deeply by showing no side effects to the patients. So, we had chosen some herbal drugs for CRC which has an activity to cure cancer. 5-fluorouracil, Curcumin, Vanillic acid, Hesperidin, Resveratrol, Fisetin, and EGCG are the drugs that can treat cancer which allowed for to study of the interaction with the proteins 1C5Y, 1N8Z, 3BBF, 1UEA, 3BBB, and 6VTC. The result of this study was we found that molecular docking properties and ADMET properties of the drugs. It helps make formulations for CRC based on the property of the drug, as the results mentioned above tables based on different properties. These results will help to improve the drug study for the CRC and its activity, by less cost, without manpower and clinical trials.

Abbreviations

EGCG: Epigallocatechin gallate
CRC: Colorectal cancer
RCSB PDB**:** RCSB Protein Data Bank
SLN: Solid Lipid Nanoparticles

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Conflicts of Interest:

The authors declare no conflict of interest.

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