ORIGINAL ARTICLE

Identification of Anticancer Potential of Phytoconstituents of *Cuminum cyminum* Effective against Hepatocellular Carcinoma Using Computational Approaches

Hina Majid Khan[1], Bushra Bibi[1], Erum Dilshad[1*], Rehana Rani[2]

ABSTRACT

Objective: To explore anticancer agents from *Cuminum cyminum* against hepatocellular carcinoma.

Study Design: In-silico approaches using computational tools to determine the anticancer potential of phytoconstituents of cumin against hepatocellular carcinoma.

Place and Duration of Study: This study was conducted from May 2021 to January 2022 at the Department of Bioinformatics and Biosciences of Capital University of Science and Technology, Islamabad, Pakistan.

Materials and Methods: In this study, bioactive compounds of *Cuminum cyminum* representatives of Flavonoids, Phenolic acids, Terpenes, and Glycosides were selected to determine the anticancer potential of these ligands using an in-silico approach. Virtual screening of these ligands was carried out against the drug target, which was Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2). To identify novel anticancer bioactive compounds of *Cuminum cyminum* as potential inhibitors of VEGFR-2, Lipinski rule of five, Pharmacokinetic properties, and molecular docking were performed. Lipinski's rule of five and the Pharmacokinetic properties of ligands were studied through the pkCSM tool. Auto-dock performed molecular docking.

Results: From these selected compounds, four ligands showed themselves as hit compounds. Among them, Quercetin was selected as the lead compound in this research against VEGFR-2 because it showed a -10.14 kcal/mol binding score and showed more active results with less toxic effects than the standard drug, which was Lenvatinib. All ligands-protein interaction visualization analyses were performed by PyMol molecular visualization tool and Discovery tool.

Conclusion: Quercetin was identified as a lead compound that should be explored as a drug candidate in wet lab analysis to validate its efficacy for the treatment of HCC.

Keywords: *Cuminum cyminum, Hepatocellular Carcinoma, Molecular Docking, Drug Discovery. VEGFR-2.*

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[1]*Department of Bioinformatics and Biosciences*
Faculty of Health and Life Sciences
Capital University of Science and Technology (CUST), Islamabad, Pakistan
[2]*Department of Life Sciences*
Abasyn University, Islamabad, Pakistan

Correspondence:
Dr. Erum Dilshad
Associate Professor, Bioinformatics and Biosciences
Capital University of Science and Technology (CUST), Islamabad, Pakistan
E-mail: dr.erum@cust.edu.pk

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Introduction

Hepatocellular carcinoma (HCC) is commonly known as primary liver cancer and is globally considered the sixth most common cancer and the third leading cause of cancer mortality, with 905,700 cases and 830,200 deaths reported in 2020.[1] Due to poor prognosis, only 12.7% is three-year survival rate and the average time of survival in HCC is only nine months.[2] According to an international organization Cancer Today, 80% of cases of HCC were reported in Asia and sub-Saharan Africa; out of 826,000 cases of liver cancer, 661,000 cases were due to hepatocellular carcinoma.[3-4] There are non-specific

and clinical symptoms of HCC. In non-specific symptoms, nausea, weight loss, fever, abdominal pain, and anorexia are frequently present in HCC patients. Clinical symptoms are liver inflammation, jaundice, peripheral oedema, ascites, and gastro-bleeding.[5] Most common risk factors for HCC are hepatitis B and C, chronic alcohol intake, aflatoxins exposure, and non-alcoholic fatty hepatic diseases.[6] Approximately 50% of cases of hepatocellular carcinoma are closely associated with Hepatitis B infection, and 25% of HCC cases are related to Hepatitis C infection.[7]

HCC is a highly vascularized cancer. It's very challenging to decide the best treatment for HCC as it involves changes in multiple signaling pathways and complex pathophysiology. Two treatment options for HCC are curative and palliative treatments. Curative treatments include liver transplant, percutaneous ablation, and surgical resection. These treatments are useful for the early stages of HCC. But early diagnosis of HCC is very challenging as it involves the analysis of angiogenesis at the molecular level. Moreover, these treatment methods are not cost-effective. Palliative treatments include radioembolization, trans arterial chemoembolization, and systemic and molecular targeted therapies. Patients who are in advanced stages of HCC prefer to use these treatments option because it improves overall survival rate and quality of life. Curative treatment is effective for a small group of patients as it involves external and internal factors such as the size of the tumor, organ shortage, metastatic nature of HCC, liver function status, and late diagnosis. But palliative treatments showed systemic toxicity, unpredictable therapeutic outcomes, cancer recurrence and impaired liver function. Thus, available treatments of HCC do not show satisfactory survival rate so there is a need to look for better treatment options. In most of the cases, HCC is diagnosed at later stages requiring combination and molecular targeted therapies for better therapeutic results. In the past few years, targeted therapies have gained much attraction to treat most of the cancer.[8,9]

In this research study, secondary metabolites of cumin have been explored to identify a new compound effective against HCC. The new compound is identified on the basis of the ability of a compound to inhibit the VEGFR-2 in the HCC case. The underlying mechanism for the identification of new compounds is based on applying primary and secondary filters for screening out the hit compounds. Then analyzing, the docking score and protein-ligand interactions of hit compounds for the selection of lead compound. In primary filters, Lipinski's rule of five is used for assessing the drug ability of selected compounds. Then secondary filter was applied to the compounds which complied with the Lipinski rule of five. In the secondary filter pharmacokinetic properties of compounds were examined using pkCSM tool. At the end, docking results of the remaining compounds were compared for the selection of lead compound effective for VEGFR-2 inhibition in HCC.

Different computational tools are available for the in-silico analysis of secondary metabolites for identifying a new compound with therapeutic potential. This research study used the Zinc database, Protein Data, pkCSM tool, AutoDock, and Discovery studio. The timeline and cost of developing a new drug is significantly increasing with the increasing complexities of diseases. There is need to use computational approaches for improving the efficiency of drug discovery process. According to recent studies, 90 percent of the clinical trials in drug discovery failed due to incompetent target validation.[10] Rejection of new drugs in the clinical phase II and III is associated with the unexpected outcomes and side effects of the potential drug. It significantly increases the attrition rate in clinical trials which is usually linked with the unpredicted pharmacokinetic properties of the compound. Use of computational approaches for the identification of potential interaction of ligand with the protein reduces the risk of error in the pre-clinical trials.[11] It offers a time and cost – effective approach for the rapid screening and optimization of toxicity and pharmacokinetic properties of lead compound.[12] It complement the experimental approaches in the drug discovery. Thus, computational prediction tools are preferred for pre-clinical trials for improving cost, time effectiveness and reducing the risk of failure in the clinical trials.[13]

HCC occurs because of deregulation of molecular

pathways which led the foundation to identify the new targeted therapies. Molecular pathways which play a significant role in HCC development have been described in recent studies. These pathways help in chemoprevention of HCC. Deregulated signalling pathways in HCC includes, EGFR/RAS/MAPK, VEGF/PDGF, Wnt/β-catenin, IGF and PI3K/AKT/mTOR pathways.[14] The most prominent pathway which is involved in the pathogenesis of HCC is the receptor tyrosine kinase pathway which mainly includes vascular endothelial growth factor receptor-2(VEGFR-2). This pathway is associated with cell proliferation and angiogenesis causing the development and spread of HCC.[15] VEGF is considered as angiogenic factor and responsible for angiogenesis. VEGF levels are normal in a healthy person but it shows elevated levels in cancer patients.[16] High levels of vascular endothelial growth factor (VEGF) are expressed in about 91% of hepatocellular carcinoma patients.[17,18] It usually acts on VEGFR-2 and cause initiation of cancer stem cells and development of blood vessels which are responsible for invasion, growth and spread of tumor.[19] So, signaling cascades of VEGF/VEGFR-2 are responsible for cell division, migration and permeability of vascular endothelial cells.[20] Overexpression of VEGFR-2 in HCC patients were reported in several studies. In HCC patients, high levels of VEGFR-2 with advanced stages of disease, more vascular invasion and large tumor size were found by Matsui et al.[21] VEGFR-2 is a prognostic factor and its high level are responsible for more vascular invasion and poor survival rate in HCC patients.[22] Thus, in this study, VEGFR-2 was selected as targeted protein as it involves in the deregulation of VEGF/VEGFR-2 pathway and plays a great role in pathogenesis of HCC. Very few targeted drugs are available in market to treat deregulation of VEGFR-2 pathway. It has been reported that available drugs show low response rate, high toxicity, and less modest anticancer activity. So, it is very necessary to look for more targeted drugs which can easily treat alterations in VEGF/VEGFR signalling pathway and shows more effectiveness with less toxicity.[23]

Some targeted drugs like Lenvatinib and Soraferinib are the only drugs which solely targets the VEGFR-2.[24] Consequently, anti-angiogenic drugs which blocks the VEGF/VEGFR-2 cascade gives a novel target for the clinical treatment of HCC and also showed safety and efficacy in HCC.[25,26] In HCC patients, expression of VEGFR-2 gains clinical importance because of its susceptibility to targeted therapy.[26]

Drugs derived from plants can be used for the treatment of different diseases because of their bioactive compounds which play important role in regulating body functions and their pharmacological potential is used for the preparation of new drugs.[27] Plants with medicinal activities are considered as backbone for traditional medicine so it means that medicinal plants are used by more than 3.3 billion populations of third world countries on regular basis.[28]

Cuminum cyminum was selected in this study for exploring its therapeutic potential against HCC as it shows many biomedical activities because of its bioactive components. *Cuminum cyminum* is a flowering plant that belongs to the Apiaceae family.[29] *Cuminum cyminum* contains terpenes, phenolic acids, glycosides, and flavonoids as major bioactive compounds. To treat variety of diseases, they are commonly used in traditional medicines. Chronic diarrhea, acute gastritis, dyspepsia, cancer and diabetes can be treated through *Cuminum cyminum*.[30] The phenolic compounds and Flavonoids are effective for human health and for curing and preventing many diseases because of their antibacterial, antioxidant, cardioprotective, anticancer, and anti-inflammatory effects. For pharmaceutical and medicinal applications, flavonoids and phenolic compounds are alternative sources with fewer side effects.[31] Cumin shows anticancer activity because it can modulate carcinogen metabolism via carcinogen-xenobiotic metabolizing phase I and phase II enzymes.[32] Based on this evidence cumin was selected in this study to explore the anticancer potential of *Cuminum cyminum* against HCC. *Cuminum cyminum* showed effective cytotoxic activity against breast cancer cell lines. Very few studies are available on exploration of bioactive compounds of cumin.[33] Based on this evidence cumin was selected in this study to explore the anticancer potential of *Cuminum cyminum* against HCC.

Lenvatinib was selected as standard drug for the

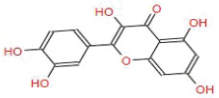
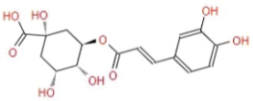
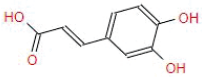
comparison with the identified lead compound because it is a FDA-approved drug and used as first line treatment for advanced stages of HCC. Moreover, it specifically targets the VEGFR-2 which is selected as targeted protein in this research study for HCC treatment.[34]

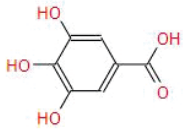
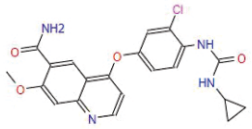
The objectives included the identification of novel, less toxic anticancer bioactive compounds of *Cuminum cyminum* as potential inhibitors of VEGFR-2, to analyze the binding conformation between targeted protein and ligands by performing molecular docking and find the best of the interacting molecules that show inhibitory effects against the targeted receptor that could be used as an anticancer agent against HCC.

Materials and Methods

In HCC, there is an overexpression of VEGFR-2, so inhibition of VEGFR-2 is a possible way to induce cytotoxic activity in tumor cells and treat hepatocellular carcinoma. So VEGFR-2 was selected as the targeted protein by literature review for determining the anticancer potential of bioactive compounds of *Cuminum cyminum* against HCC.[35] 3D structure prediction of selected targeted protein was done using RCSB PDB. Protein Data Bank is a three-dimensional database of complex molecules of living organisms, such as proteins and nucleic acids. The structure of human VEGFR-2 in complex with imidazo [1,2-b] pyridazine derivative was downloaded in PDB format. PDB ID of Vascular Endothelial Growth Factor Receptor 2 was 3VO3. Functional domains of 3VO3 were identified using the Pfam database by providing the Uniprot ID of 3VO3 as a query. Active site prediction was done using PyMol based on ligands attached with 3VO3. The active sites were predicted because it helps in setting grid parameters in the AutoDock tool for focused docking and enhances the accuracy of focused docking by decreasing the search space on the surface of the receptor. After the prediction of active sites, the targeted protein was refined for docking by removing attached ligands and hetatoms like water molecules using PyMol because structure refinement is an important step in achieving accurate results of docking. The next step was the preparation of ligands for docking. Bioactive compounds belonging to different classes including Flavonoids, Phenolic acids, Terpenes, and Glycosides were selected as ligands from *Cuminum cyminum* by a literature review. The 3D structure of ligands was downloaded in SDF format from the Zinc database.[36] The selected ligands are shown below in Table 1.

Table 1: The chemical structure and properties of selected ligands

Name	Molecular Formula	Molecular weight (g/mol)	Structure
Quercetin	C15H10O7	302.238	
Chlorogenic acid	C16H18O9	354.311g	
Caffeic acid	C9H8O4	180.159	

Gallic acid	C7H6O5	170.12	
Lenvatinib	C21H19ClN4O4	426.86	

Energy minimization of ligands was done by Chem pro software (chem 3D v 12.0.2) to enhance docking results' accuracy. The 3D structure of ligands with minimized energy was saved in pdbqt format. After that, virtual screening was done to measure the toxicity of ligands. In virtual screening, two filters were applied using the pkCSM tool, one was a primary filter called Lipinski's rule of five, and the second one was Pharmacokinetic studies of ligands. Selected bioactive compounds were further screened based on molecular docking score. Molecular docking of targeted proteins with bioactive compounds of *Cuminum cyminum* was performed using AutoDock 1.5.6 with MGL tools. In the AutoDock tool, refined 3D structure of 3VO3 in pdb format was added as a rigid macromolecule, and optimization of 3VO3 was done by adding Kollman charges. Polar hydrogen atoms to the 3D structure of 3VO3 macromolecule. The 3D structure of the ligand was added in pdbqt format.[12-36]

The ligand's structure was optimized by adding the Gasteiger charges and detecting the number of torsions in the AutoDock tool. After optimization of the macromolecule and ligand, the next step was the creation of a grid parameter and docking parameter file. The grid parameter file was created by setting the grid box size. XYZ dimensions of the grid box were set at 80x 80 x 80 with a spacing of 0.375Å. The center of x, y, and z was determined based on predicted active sites of 3VO3. For docking of 3VO3 with the ligands center of x, y, and z were set at 21.71409375, -26.0365, and -18.39740625, respectively. After setting docking parameters the next step was running AutoDock to find out the docking score of ligands.

Docking analysis was done using Discovery Studio in which interactions of ligands and targeted proteins were predicted. Discovery Studio is a comprehensive and free software that is used to model and analyze molecular structures and sequences. It has a wide range of tools that helps to visualize the data.[36] Novel lead compound was identified by screening ligands through primary and secondary filters. The best ligand with the highest binding score and the best safety profile for the target protein was identified. Drugs used for hepatocellular carcinoma were identified using the KEGG database. The identified drugs were filtered to select the most effective drug. Lenvatinib was selected as an effective drug by setting different parameters, which included physiochemical properties, pharmacokinetic properties, docking score, fewer side effects, and effective mechanism of action using PubChem, pkCSM tool, AutoDock, and KEEG database, respectively. The comparison between selected chemo-drug and proposed anticancer agents was done by comparing docking results, physiochemical properties, and ADMET properties to assess their bioavailability, drug-likeness, efficacy, and safety. This research work was carried out from May 2021 to January 2022 at the Bioinformatics lab of the Department of Bioinformatics and Biosciences of

Capital University of Science and Technology, Islamabad, Pakistan.

Results

VEGFR-2 consists of 1356 amino acid residues having a molecular weight of 151526.81 Dalton. There are total 6 functional domains of VEGFR-2 which includes 2 domains of Immunoglobulin domain- 3 (Ig-3), 2 domains of (Immunoglobulin I-set domain) I-set, VEGFR-2 Transmembrane domain(VEGFR-2-TMD) and Protein tyrosine and serine/threonine kinase domain(PK-Tyr-Ser-Thr). Protein domains are structural and functional units of a protein.[37,38] 3D structure of VEGFR-2 predicted using the Protein Data Bank is shown below in Figure 1.

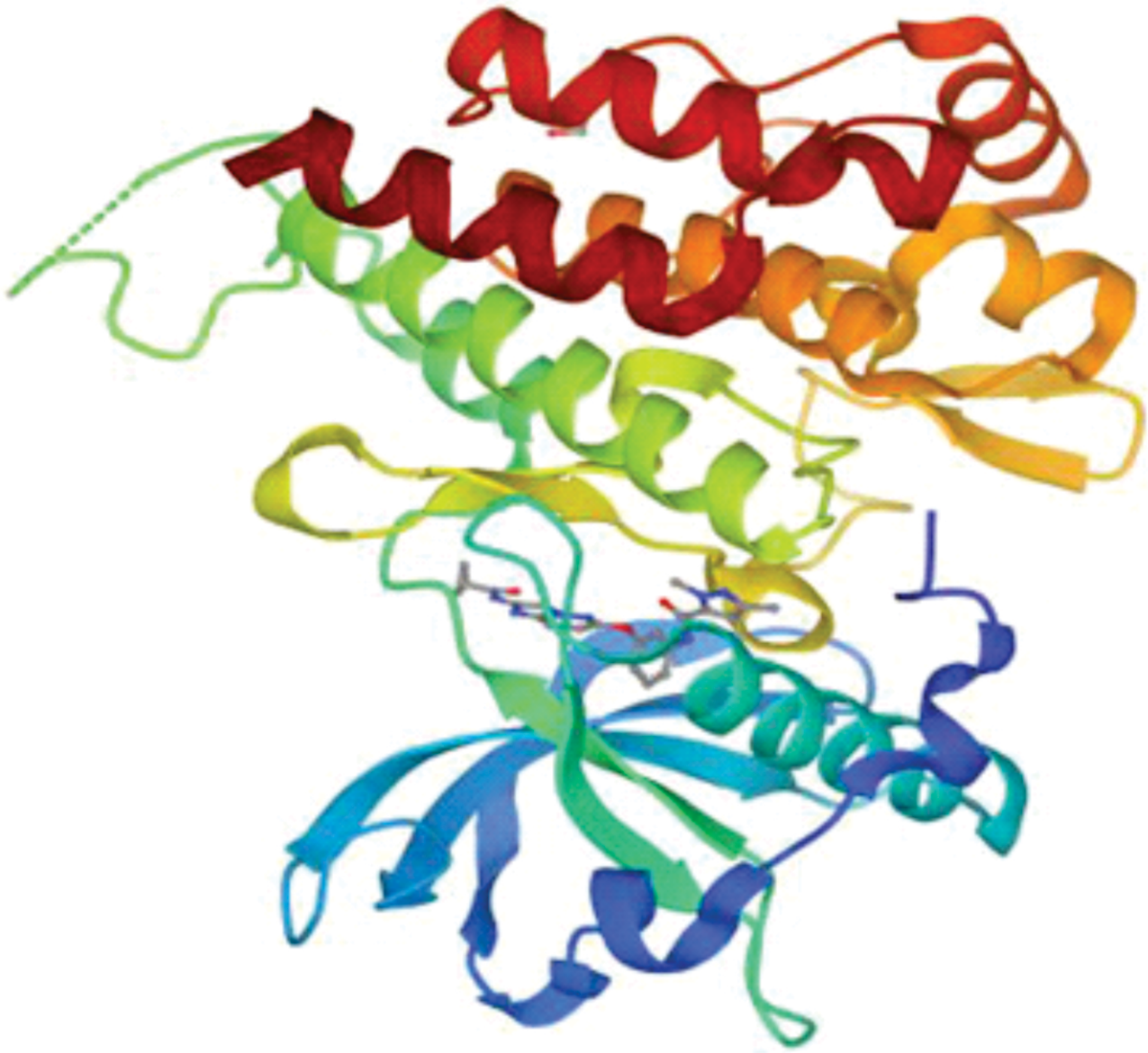


Fig 1: 3D Structure of Vascular Endothelial Growth Factor-2 in complex with imidazo[1,2-b] pyridazine derivative Retrieved from Protein Data Bank

Lipinski's rule of five (RO5) was used to evaluate the drug-likeness of a compound, to determine whether a chemical compound with certain pharmacological activity can be used as an active drug in humans that can be taken orally.[39] Below, Table 2 shows the applicability of Lipinski's rule of five on selected ligands. All ligands and Lenvatinib drugs complied with all Lipinski's rules.

Table 2: Lipinski's Rule of Five applied to Selected Ligands

Ligand	Molecular weight g/mol	LogP value	H-Bond donor	H-bond acceptor
Quercetin	302.238	1.988	5	7
Chlorogenic acid	354.311	-0.6459	6	8
Caffeic acid	180.159	1.1956	3	3
Gallic acid	170.12	0.5016	4	4
Lenvatinib	426.86	4.0719	3	5

ADMET properties of ligands and standard drugs were extracted from the pkCSM tool. The best drug candidate must have appropriate pharmacokinetic properties at a specific therapeutic dose along with adequate efficacy against a therapeutic target.[23]

ADMET properties of all ligands showed that all ligands had a good safety profile. All selected ligands were complying with all ADMET models. Quercetin has the highest value of intestinal absorption (74.9%) among all ligands. Most drugs stay in a balanced state between binding and unbinding state (Fraction unbound). Usually, drugs bind with proteins present in the serum. FU predicts the fraction of compounds that will not bind with serum proteins but present unbounded in plasma. Drug efficacy is affected by the level at which it binds with protein present in blood. If a substance shows a high FU value, then it is more effective.[40] The Fu value of Quercetin was higher than all selected ligands and the FDA-approved standard drug Lenvatinib. So more unbound fractions of Quercetin will be available for producing effect if it is taken as a drug. Chlorogenic acid, Caffeic acid, and Gallic acid showed logBB value greater than 0.3 so these ligands are not safe because they can cross the blood-brain barrier and can cause harm to the brain, whereas the logBB value of Quercetin and Lenvatinib was less than 0.3 so it is safe as it will not cross the blood-brain barrier. Metabolic properties showed that Lenvatinib may have severe adverse effects due to slow metabolism as it is an inhibitor of two isoforms CYP2C9 and CYP3A4 while Quercetin showed negative results against inhibition of CYP2C9 and CYP3A4. Among all ligands, Gallic acid showed a very high value which means it can cause toxic effects and Chlorogenic acid showed a very low value which means it will not produce desired therapeutic effects. The selected drug also showed a very low value which means it does not produce desired therapeutic effects. Quercetin and Caffeic acid both showed ideal values which are not too high or not too low. All compounds showed a negative result for the Renal OCT2 substrate model validating that they are not interfering with the normal functioning of OCT2-organic cation transporter 2 which is important for renal clearance of the drug. All ligands were satisfying the toxicity models, but Lenvatinib violated hERG I and II inhibitor models and the hepatotoxicity model. ADMET properties of selected compounds are shown in Table 3.

Table 3: The ADMET Properties of Selected Ligands and Lenvatinib Absorption Properties

Models	Quercetin	Chlorogenic acid	Caffeic acid	Gallic acid	Lenvatinib
Water solubility log mol/L	-3.005	-2.357	-1.699	-1.914	-4.201
Caco2 permeability Log Papp; log cm/s	0.286	-0.939	0.081	-0.026	0.58
Intestinal absorption %	74.9	9.123	59.008	40.154	99.133
Skin Permeability log Kp	-2.735	-2.735	-2.739	-2.737	-2.738
P-glycoprotein substrate	Yes	Yes	Yes	No	Yes
P-glycoprotein I inhibitor	No	No	No	No	Yes
P-glycoprotein II inhibitor	No	No	No	No	Yes
Distribution Properties					
VDss log L/kg	0.113	-0.724	-0.519	-0.27	0.252
Fraction unbound Fu	0.087	0.537	0.409	0.368	0.014
BBB permeability Log BB	-1.573	-1.672	-0.831	-1.424	-1.509
CNS permeability Log PS	-3.41	-4.137	-3.327	-4.131	-2.557

Metabolic Properties					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	Yes
CYP1A2 inhibitor	Yes	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes
Excretion Properties					
Total Clearance	0.555	0.314	0.558	0.625	0.213
Renal OCT2 substrate	No	No	No	No	No
Toxicity Models					
AMES toxicity	No	No	No	No	No
Max. tolerated dose	0.841	0.664	0.428	0.519	0.446
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes
Oral Rat Acute Toxicity	2.437	2.192	2.853	2.03	2.404
Oral Rat Chronic Toxicity	2.69	3.832	1.913	3.241	1.614
Hepato-toxicity	No	No	No	No	Yes
Skin Sensitisation	No	No	No	No	No
T. pyriformis toxicity	0.293	0.285	0.056	0.226	0.307
Minnow toxicity	1.328	3.393	1.652	1.938	0.999

Molecular docking of VEGFR-2 with selected ligands and the standard drug Lenvatinib was performed three times to validate the results. The results of protein-ligand docked complexes were evaluated based on the highest binding energy values because the more the binding energy is high, the conformation formed will be stable.[36] Results of three times docking showed that all ligands had good binding energy scores ranging from -10.14 to -6.06 kcal/mol against vascular endothelial growth factor receptor-2, and among all ligands, Quercetin had the best binding energy score than ligands and Lenvatinib, which means it formed a more stable complex with the VEGFR-2 and more potent to inhibit the expression of VEGFR-2. The ligand efficiency value of Quercetin and Chlorogenic acid was better than Caffeic acid and Gallic acid. Ligand efficiency tells about the ability of the ligand to bind with the macromolecule, as shown below in Table 4.

Table 4: The comparison of the docking score of ligands and Lenvatinib

Number of Dockings	Compounds	Quercetin	Chlorogenic acid	Caffeic acid	Gallic acid	Lenvatinib
1	Binding Score	-10.14	-10.04	-6.69	-6.06	-9.11
2	kcal/mol	-10.13	-9.79	-6.68	-6.06	-9.29
3		-10.14	-9.9	-6.72	-6.07	-9.46
1	Ligand	-0.46	-0.4	-0.51	-0.51	-0.3
2	efficiency	-0.46	-0.39	-0.51	-0.51	-0.31
3		-0.46	-0.4	-0.51	-0.51	-0.32
1	Inhibition	36.74	43.86	12.47	36.07	209.99
2	constant	37.53	66.83	12.67	35.59	155.4
3	nM	36.93	54.93	11.9	35.83	115.6
1	Torsion	0.3	1.79	0.89	0.3	1.79
2	energy	0.3	1.79	0.89	0.3	1.79
3	kcal/mol	0.3	1.79	0.89	0.3	1.79
1	Ref RMSD	34.88	38.13	40.6	40.92	45.28
2	A°	34.88	41.12	40.46	40.91	44.69
3		34.88	38.02	40.59	40.91	34.58

Quercetin showed the best binding energy ranging from -10.13kcal/moL to -10.14 kcal/mol quite better than Lenvatinib. The mean values of the binding score are shown below in Table 5.

Table 5: Mean binding score of ligands and Lenvatinib

Ligands	Mean Binding scores kcal/mol	Mean Inhibition constant	Mean Ligand efficiency
Quercetin	-10.14	37.07	-0.46
Chlorogenic acid	-9.91	55.21	-0.39
Caffeic acid	-6.69	12.35	-0.51
Gallic acid	-6.06	35.83	-0.51
Lenvatinib	-9.29	160.33	-0.31

The standard deviation errors observed in binding energies of all docked results were 0.01, 0.13, 0.02, 0.007, and 0.17, respectively. Ligands and Lenvatinib interactions with the target protein were shown in Table 6 and these interactions were analysed by Discovery Studio. Chlorogenic acid, Caffeic acid, and Gallic acid made 5, 3, and 2 hydrogen bonds with the targeted protein VEGFR-2, respectively. Quercetin, Chlorogenic acid, Caffeic acid, and Gallic acid consist of 15, 16, 9, and 7 carbon atoms, respectively. 32 active site residues were predicted in VEGFR-2. Chlorogenic acid, Caffeic acid, and Gallic acid showed hydrophobic interactions with 5, 3, and 3 amino acid residues of active sites of targeted protein VEGFR-2, respectively. Quercetin made 7 hydrogen bonds at LEU840, GLU917, CYS1045, ASP1046, CYS919, CYS919, and GLU885 with a distance of 2.80, 2.56, 3.46, 2.75, 2.89, 1.66 and 3.31 angstrom and showed hydrophobic interactions with 8 amino acid residues of the VEGFR-2 where the ligand binds. While Lenvatinib binds active residues of the target protein by forming 5 hydrogen bonds at CYS919, CYS919, ARG842, ARG1051, and ARG1051 with bond distances of 2.98, 1.84, 3.08, 3.19, and 3.18 Angstrom and formed 8 hydrophobic interactions with active residues of VEGFR-2.

Table 6: Ligands showing hydrogen and hydrophobic interactions with VEGFR -2

Ligand	No. of hydrogen bonds	Interacting amino acids in H-Bonding	H-Bonding distance(Å)	Hydrophobic interactions	Hydrophobic Interaction distance(Å)
Quercetin	7	:UNL1:O A:LEU840:O	2.80	A:ALA866 - :UNL1	4.69
		:UNL1:O - A:GLU917:O		A:VAL916 - :UNL1	
				A:VAL899 - :UNL1	4.73
		:UNL1:O - A:CYS1045:SG:B	2.56	A:CYS1045 - :UNL1	4.67
		:UNL1:O - A:ASP1046:O	3.46	A:PHE1047 - :UNL1	4.57
		:UNL1:O - A:CYS919:O	2.75	A:VAL848 - :UNL1	5.47
		A:CYS919:HN - :UNL1:O	2.89	:UNL1 - A:LEU1035	4.70
				:UNL1 - A:LEU840	
		:UNL1:O - A:GLU885:OE2	1.66		4.56
					4.43
			3.31		
Chlorogenic acid	5	A:ASP1046:HN - :UNL1:O	2.23	A:VAL916 - :UNL1	3.63
				A:VAL899 - :UNL1	
		:UNL1:O - A:ASP1046:OD2	3.35	A:LYS868 - :UNL1	5.38
				:UNL1 - A:LEU1019	4.76
		:UNL1:O - A:ILE1025:O	2.76	A:HIS1026 - :UNL1	5.46

		:UNL1:O - A:ILE1025:O	2.97		5.06
		:UNL1:O - A:CYS1045:SG:B	3.70		
Caffeic acid	3	:UNL1:O - A:VAL899:O	3.38	A:VAL899 - :UNL1	5.36
				A:VAL916 - :UNL1	
		:UNL1:O - A:ILE1044:O	2.80	A:LYS868 - :UNL1	4.20
		:UNL1:O - A:CYS1045:SG:B	3.76		4.71
Gallic acid	2	:UNL1:O - A:VAL914:O	3.26	:UNL1 - A:VAL899	4.73
				:UNL1 - A:VAL916	
		:UNL1:O - A:ASP1046:O	3.04	A:CYS1045 - :UNL1	4.40
					5.09
Lenvatinib	5	:UNL1:O - A:CYS919:O	2.98	:UNL1 - A:VAL848	4.67
				A:ALA866 - :UNL1	3.46
		A:CYS919:HN - :UNL1:O	1.84	:UNL1 - A:LEU1035	4.96
		:UNL1:O - A:ARG842:O	3.08	A:LEU1035 - :UNL1	5.38
		:UNL1:N - A:ARG1051:NH2	3.19	A:PHE1047 - :UNL1	4.94
		A:ARG1051:NE - :UNL1:N	3.18	:UNL1:C - A:PHE1047	3.74
				A:PHE1047 - :UNL1	4.65
				A:ARG1051 - :UNL1	4.56

After applying Lipinski's rule of five and ADMET properties, all ligands showed good safety profiles, but based on binding score, Quercetin was selected as the lead compound as it had the highest binding score against VEGFR-2 among all ligands. Quercetin strongly interacted with VEGFR-2 as it has more hydrogen bonds which help it to make the stable confirmation as compared to other ligands, as shown below in Table 7. It means that it can inhibit VEGFR-2 more effectively and possess strong anticancer properties. The anticancer effect of Quercetin has been reported in many experimental studies. Antitumor activity of Quercetin was reported by Tang et al. in 2020[41] as it decreases the formation of blood vessels by targeting VEGFR-2 pathway, which is responsible for tumor growth by causing angiogenesis in prostate and breast cancer. Quercetin also showed its potential to stop angiogenesis in drug-resistant cells, which makes it superior to antitumor drugs.

Table 7: Comparison of toxicity models of lenva tinib and quercetin

Model Name	**Predicted Values**	
	Lenvatinib	**Quercetin**
AMES toxicity	No	No
Max.tolerated dose(human) (log/mg/kg/day)	0.446 log/mg/kg/day	0.841
hERG I inhibitor	No	No
hERG II inhibitor	Yes	No
Oral rat acute toxicity (LD50) mol/kg	2.404 mol/kg	2.437
Oral rat chronic toxicity (LOAEL) (log/mg/kg_bw/day)	1.614 log/mg/kg_bw/day	2.69
Hepatoxicity	Yes	No
Skin sensitization	No	No
T. pyriformis toxicity (log ug/L)	0.305 log ug/L	0.293
Minnow toxicity(mM)	-0.087 mM	1.328

The Maximum tolerated dose of Lenvatinib is less than the selected lead compound "Quercetin" which highlights that the bioactive compound is ahead in the safety of Lenvatinib. It is evident from Table 7 that Lenvatinib showed itself as an hERG II inhibitor. Mostly hERG I/II inhibitors are withdrawn from the pharmaceutical market due to their adverse effects and as such types of drugs are responsible for causing QT syndrome. So it is clear here as well that Quercetin has a high safety profile as compared to reference drugs because Quercetin showed negative results in the case of both hERG I and II inhibitor models. LOAEL predicted value of Lenvatinib is less than Quercetin which shows its potency to be more toxic than bio compound. The hepatotoxicity model indicates that Lenvatinib is toxic to the liver and can cause injury to the liver as it showed a positive result in the hepatotoxicity model.

The 3D visualization of docking complexes showed that Quercetin as a lead compound and Lenvatinib as a standard drug binds at the same active sites of selected protein VEGFR-2 as shown below in Figure 2-4 respectively. Quercetin strongly interacted with active sites of VEGFR-2 and makes more stable conformation as compared to standard drugs.

The molecular docking score of Lenvatinib with

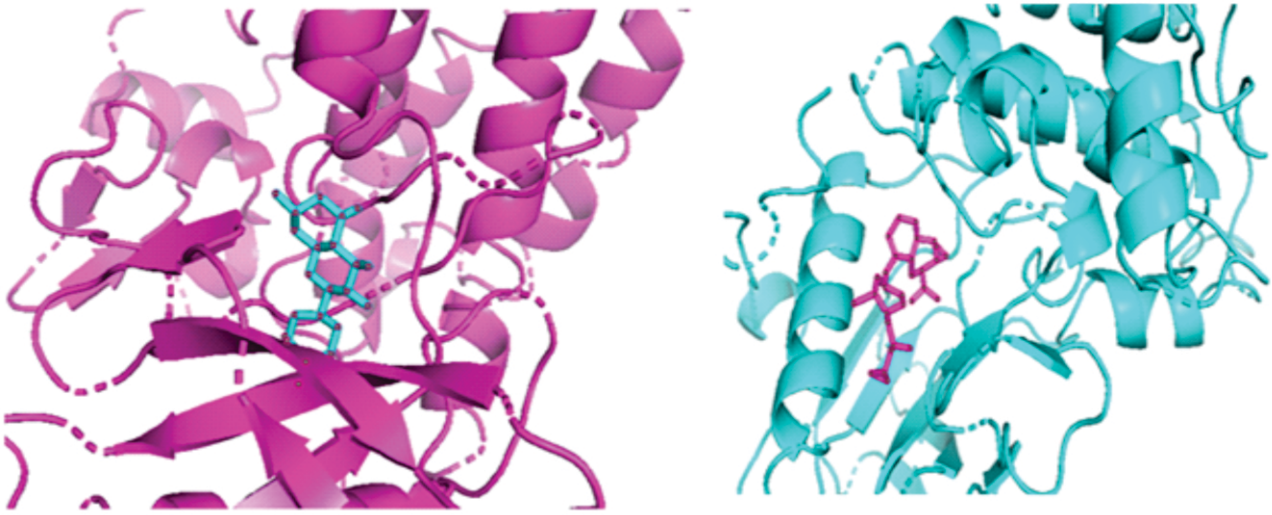


Fig 2: Interaction of quercetin with VEGFR-2 (A) and interactions of lenvatinib with VEGFR 2(B)

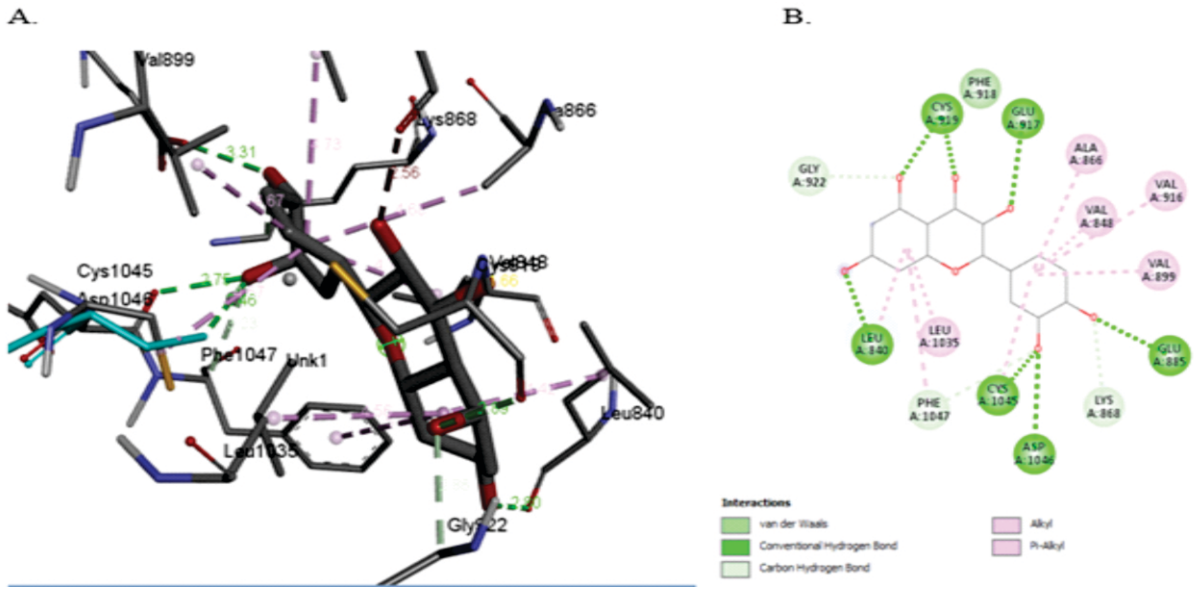


Fig 3: The binding interactions of Quercetin with active sites of VEGFR-2. Green dotted lines show hydrogen bonding and Purple dotted lines represent alkyl bonding

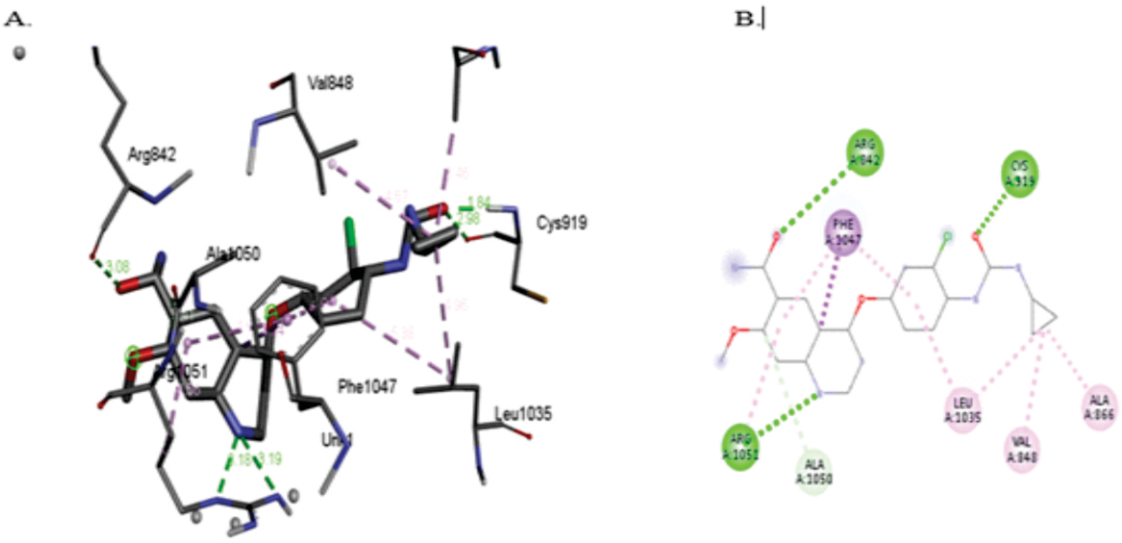


Fig 4: The binding interactions of lenvatinib with active sites of VEGFR-2. Green dotted lines show hydrogen bonding, and purple dotted lines represent alkyl bonding

VEGFR-2 protein was less than the docking score of Quercetin. The highest score of Quercetin indicates that Quercetin has more stable conformation with VEGFR-2 as compared to Lenvatinib. Primary and secondary filters also showed that the safety profile of Quercetin was best than the standard drug. These results validate the stability and efficacy of Quercetin over available marketed drugs.

Discussion

The motive of the present research was to identify bioactive compounds of *Cuminum cyminum* which could act as anticancer agents in HCC. Previously, it has been reported in the literature that quercetin possess anticancer activity against prostate and breast cancer. For this purpose, bioactive compounds of cumin were selected after performing data mining studies on literature databases. These ligands belong to different classes of secondary metabolites, such as flavonoids, phenolics, terpenes, and glycosides. In HCC, VEGFR-2 was selected as the target protein. To find out the lead compound, primary and secondary filters were applied, and docking was done against targeted proteins. The structures of all ligands were downloaded from the zinc database, and 3D structures of targeted protein structures were available in PDB. Drug likeliness of compounds was studied by using primary and secondary filters. Lipinski's rule of 5 was used as the primary filter, and pharmacokinetics properties were used as a secondary filter. The docking procedures were performed using the AutoDock tool. The results were visualized using PyMol and ligand-protein interactions were analyzed through Discovery Studio. After a detailed analysis of physicochemical properties, Lipinski's RO5, ADMET properties, and a binding score of ligands, Quercetin, with the best binding score and the best safety profile, were selected as the lead compound against all targeted receptor proteins selected in this research work. Virtual screening results, physiochemical properties, and pharmacokinetics properties of Quercetin were compared with Lenvatinib. Thus, based on these results, it was found that Quercetin shows better binding affinity to respective protein targets and shows less toxicity than standard drugs. Previously, there are reports showing the anticancer and anti-inflammatory role of quercetin in liver cancer.[42] It is the first-time cumin's therapeutic potential has been explored against HCC using computational approaches. The results of this study are beneficial for the development of targeted therapy for HCC. The development of new drugs will improve the management of HCC for the general public. However, this study focuses on exploring the therapeutic potential of bioactive compounds of medicinal plants, which will assist the scientific community in looking for cost-effective and safe treatment methods.[43]

Conclusion

Quercetin has been screened as a lead compound from *Cuminum cyminum* showing efficacy against receptor protein VEGFR-2 in case of liver cancer. However, further studies and clinical trials should be performed for take this drug candidate to the advanced level of treatment.

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