Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 2s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Molecular Docking Study on Cholangiocarcinoma Target Protein with Natural Plant Derived Ligands for Potential Therapeutic Applications

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Cite this paper as: David Annaraj P, Muhammed Ameen O, Ansiya Abbas, Muhammed Sadik KM, (2025) Molecular Docking Study on Cholangiocarcinoma Target Protein with Natural Plant Derived Ligands for Potential Therapeutic Applications, *Journal of Neonatal Surgery*, 14 (2s), 202-220

ABSTRACT

Cholangiocarcinoma (CCA) is an aggressive and malignant form of bile cancer. Its late detection, limited therapeutic options, and low survival chances poses major clinical challenges. Receptor Interacting Protein Kinase 1 (RIPK1) is a mediator of cell death and inflammatory responses in the body. With its ability to promote cancer by creating a cancer microenvironment, RIPK1 has recently gained attention, as a potential target for cancer treatment. Tumor Necrosis Factor (TNF) is what activates the RIPK1, and induces the necroptosis pathway. This study focuses on molecular docking techniques to identify phytochemicals thar are potential inhibitors of RIPK1 and TNF, by comparing their binding affinity. The results reveal that Withanolide, Ginkgolide, and Silymarin demonstrated strongest interaction with RIPK1. Withanolide being the best among these to bind with TNF as well. Drug-likeness, ADME, Rule of five assessments were carried out to study the pharmacokinetic properties of those phytochemicals to further confirm their effects. The findings suggest that these phytochemicals could serve as potential inhibitors of RIPK1, offering a natural and targeted approach for CCA therapy. Nonetheless, additional laboratory and animal studies are needed to confirm their anticancer effects, assess potential toxicity, and better understand how they work. This study shows the importance of integrating computational drug discovery approaches with natural compound research to develop novel and effective therapeutic strategies for cholangiocarcinoma

Keywords: *Hepatic cancer, Cholangiocarcinoma, Molecular docking, RIPK1, Natural ligands, Phytochemicals, Drug discovery*

1. INTRODUCTION

Cholangiocarcinoma (CCA), a malignancy that affects the bile ducts, still presents significant clinical problems due to its deceptive onset and frequently poor prognosis. CCA is considered one of the most fatal gastrointestinal cancers with limited treatment options and a tendency for late diagnosis; its progressively growing global frequency is alarming considering its usually high death rates (Mizuno et al., 2021). The location of the tumor within the biliary tree defines the classification of CCA into intrahepatic and extrahepatic subtypes; extrahepatic CCA results from the liver parenchyma and is further split depending on their distance from the liver hilum. A difference that aids therapy and prognosis assessment, intrahepatic CCA develops inside the liver parenchyma while extrahepatic CCA is further separated into proximal and distal types depending on their proximity to the liver hilum (Gonzalez et al., 2020).

The etiology of CCA is multifarious and complex. Chronic liver diseases including cirrhosis and infections with chronic hepatitis B and C greatly increase the likelihood of developing this malignancy (Klein et al., 2020). These linkages underline the crucial role environmental and viral agents perform in conjunction with genetic predispositions to produce CCA (Thanan et al., 2019).

Though understanding of CCA's biology has progressed, symptoms can present late, which delays diagnosis and, thus, less favorable consequences. Common clinical symptoms are jaundice, pruritus, stomach pain, and weight loss; these symptoms, however, are occasionally nonspecific and could be mistaken for other gastrointestinal disorders. By the time of diagnosis, many patients have advanced-stage disease, which lessens the usefulness of curative surgical treatments such as liver transplants or resections. Just 20% of patients are considered candidates for surgical resections due to the aggressive character of the tumor and late presentation (Misra et al., 2022).

Managing CCA is challenging usually necessitating a multidisciplinary strategy including surgery, chemotherapy, radiation treatment, targeted therapy, immunotherapy, and palliative care. Although it is only applicable to a small percentage of patients, surgical excision is still the only curative treatment available primarily depending on the location of the tumor and degree of disease at presentation. Although the general response rates are modest, chemotherapy with systemic medicines such as gemcitabine and cisplatin are the standard of treatment for advanced patients; this has shown some success in extending survival. Especially for specific physiologically driven subtypes of CCA, new studies have underscored the viability of immunotherapies and molecularly targeted treatments. For example, the identification of actionable mutations can assist in creating tailored treatment programs (Shindoh et al., 2022).

As our knowledge of cholangiocarcinoma (CCA) expands, receptor-interacting protein kinase 1 (RIPK1) has gained increasing importance in cancer research. RIPK1 plays a fundamental role in determining cell fate, functioning as a molecular switch that directs cells toward either survival or death. Both necroptosis and programmed cell death (apoptosis) as well as tissue homeostasis depend on RIPK1 all through appropriate development. Often considered a "clean" and controlled type of cell death, apoptosis is necessary for forming tissues and eliminating unwanted cells throughout development (Green, 2011). Conversely, (Galluzzi et al., 2016) describe necroptosis, as an inflammatory kind of cell death defined by cellular expansion followed by rupture. Activated by RIPK1, this pathway leads to the formation of the necrosome complex, which develops with the involvement of RIPK3 and Mixed Lineage Kinase Domain-Like Pseudokinase (MLKL), ultimately resulting in the breakdown of the cell. Maintaining normal tissue homeostasis and growth depends on the exact balance between death and necroptosis, mediated in major part by RIPK1.

In cancer conditions, however, this delicate balance can be disrupted. Many cancers can be caused by mutations or altered expression levels of RIPK1 and other death regulators, therefore enabling cancer cells to escape normal biological constraints and proliferate uncontrollably (Han et al., 2011). Moreover, RIPK1 has been connected to support of mechanisms outside of simple cell death. It is well known to increase inflammation, a main actor in tumor development and metastasis (Koo et al., 2020).

Given RIPK1's central role in cancer formation, aiming at RIPK1 has become a reasonable treatment strategy. Many approaches under study presently seek to regulate RIPK1 activity with effects related to cancer treatment. For some cancer models, RIPK1 inhibitors have showed promise in preclinical studies by effectively stopping necroptosis and decreasing tumor growth (Dixon et al., 2017).

Moreover, the bioactive molecules found in plants phytochemicals have drawn a lot of attention since they could help to prevent many diseases, particularly cancer, and enhance human health. These naturally occurring compounds, commonly found in fruits, vegetables, grains, and other plant-based foods, offer numerous health benefits due to their diverse chemical compositions and biological properties. Strong antioxidant properties and capacity to neutralize free radicals' unstable molecules prone to damage cellular components and aggravate chronic diseases including cancer allow phytochemicals to be quite useful (Scalbert et al., 2005).

Apart from their antioxidant properties, several phytochemicals demonstrate various ways of improving health. Some contain anti-inflammatory properties, which would help reduce the incidence of many cancers and cardiovascular diseases as well as other chronic diseases linked with prolonged inflammatory states (Minihane et al., 2015). Perhaps having significant roles in cancer prevention strategies, other phytochemicals may interact with DNA, influence cell signaling pathways, and alter enzyme function (Wojdyło et al., 20211). Diets heavy in fruits and vegetables, which abound in phytochemicals, have been related in many epidemiological studies to a reduced risk of several cancers, including lung, colon, breast, and prostate cancers (Aune et al., 2017). Though the exact molecular mechanisms by which phytochemicals exert their anti-cancer effects are yet under research, several pathways have been identified including the induction of death in cancer cells, inhibition of cancer cell proliferation, suppression of angiogenesis, and modulation of carcinogen metabolism (Karmas et al., 2020).

Moreover, under investigation are potential cancer treatment drugs based on phytochemicals. Since various phytochemicals show abilities to inhibit tumor development and metastases, preclinical studies on some of them show promise (Khan et al., 2018). Although much more in-depth research is required to determine their safety and effectiveness in humans, it is relatively safe to say that, incorporating phytochemicals into treatment plans can serve as a supportive approach alongside traditional cancer therapies.

Molecular docking has become an important computational tool in drug development due to its ability to predict interactions between target molecules, greatly reducing the need for clinical trials, saving both time and resources. This approach mimics the "lock and key" paradigm, in which the ligand (key) fits the binding site of the receptor (lock), therefore causing a biological reaction (Kitchen et al., 2004). The two main advantages of molecular docking technique are its effectiveness and affordability. It enables quick screening of large chemical libraries, saving time and cost to find viable treatment options (Shoichet, 2004).

Molecular docking is not exclusive to binding affinity predictions, it also identifies key molecular interactions. This allows researchers to refine lead compounds for increased potency, selectivity, and fewer side effects (Verdonk et al., 2003). Since

it can predict the binding strength and spatial orientation of ligands, molecular docking is an essential part of drug discovery. These discoveries enable researchers to accurately modify the structure of lead compounds, enhancing their ability to interact with target receptors (Bohacek et al., 1996).

Additionally, drug repurposing—the process of finding new uses for already-approved medications—is showing molecular docking to be a promising tool. Researchers can find novel treatment options without starting from scratch through understanding how well-known medications bind to various receptors (Ashburn & Thor, 2004).

Docking techniques aid in personalized medicine and treatment, though more genetic information is required from individual patients (Deng et al., 2015).

Recent technological advancements has drastically improved accuracy of predictions due to enhanced algorithm, better scoring techniques, and processing power (Ferreira et al., 2010). Combining docking with molecular dynamics can further help scientists to draw a complete picture of how medications and receptors interact, in real time (Zhou et al., 2020).

Combining information about plant-based compounds, RIPK1 signaling pathways, cholangiocarcinoma, and modern docking tools provides a novel, multidisciplinary approach to cancer treatment. Cholangiocarcinoma (CCA) is still difficult to treat because it is often discovered too late, can be tough to control, and in general has a poor prognosis. However, RIPK1's crucial function in determining cell fate opens up great possibilities for cutting-edge treatments that might make a real difference. Simultaneously, focusing for the health benefits of phytochemicals could be a complementary approach to therapy and cancer prevention. Finally molecular docking takes the fore stage in drug development since it provides a strong framework for the identification and improvement of new treatments targeted at specific molecular profiles, as science advances, improved diagnosis, targeted treatments and at last a better knowledge and widely survival possibilities outcome for persons affected with this aggressive disease optimism (Lie et at.,2019).

Figure 1 shows a schematic representation of the necroptosis pathway, a programmed mode of necrotic cell death, in juxtaposition with the apoptotic pathway. It highlights the key molecular players and interactions involved, starting from TNF and TRAIL receptors to the formation of the necrosome complex (RIPK1, RIPK3, MLKL) and the ultimate disruption of cellular integrity. Mitochondrial dysfunction, calcium influx, ROS production, and inflammatory responses are shown as crucial contributing factors (Kanehisa et al., 2000

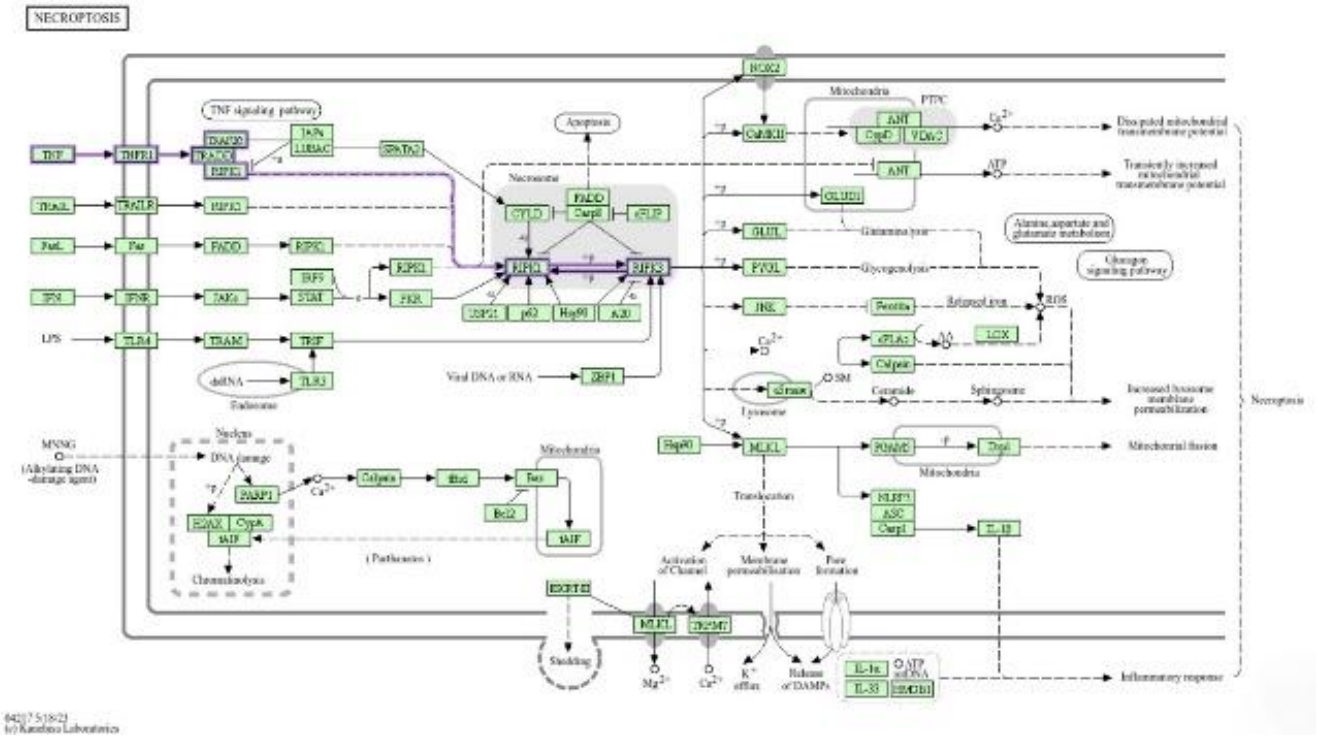

Figure 1-RIPK1 pathway in Homo sapiens

2. REVIEW OF LITERATURE

(Khan and colleagues 2019), The molecular route of cholangiocarcinoma, a very aggressive tumor of the bile ducts, is investigated in this work together with possible therapy targets. The writers go over important epigenetic changes, genetic alterations, and signaling systems linked to tumor development. Although, interesting treatment choices, they draw attention

to the developments in targeted medicines like immune checkpoint inhibitors, IDH1/2 inhibitors and FGFR inhibitor. This study underlines how important precision therapy is to raise cholangiocarcinoma patients' outcomes.

(Shlomai et al. 2019)discuss the pathways by which the hepatitis B virus (HBV)drives liver cancer including cholangiocarcinoma that are probe in this work. The authors show how HBV embrace into the host genome to cause oncogene activation and genomic instability. They also proceed how HBV proteins including HBX might disturb tumor suppressor channels .The results emphasize the importance of focused treatments meant to stop HBV driven carcinoma genesis.

(Roca suarez etal. 2018), This study indicates how viral infections affect liver cancer development including cholangiocarcinoma. The authors go over how direct genetic changes ,immune evasion, and chronic inflammation cause carcinoma genesis from hepatitis B and C viruses. They draw attention to current developments in the antiviral treatments as well as their possible lowering of liver cancer risk .The results strains the need for early virus detection and the treatment for providing hepatobiliary tumors.

(Wiskrichen and Tacken 2016), The path of liver illnesses, including cholangiocarcinoma, is founded in this review regarding hepatic fibrosis. The molecular pathways causing fibrosis such as TGF-β signaling and hepatic stellate cell activation - are detailed by the authors. They investigate over possible antifibrosis and block liver cancer. The results highlight fibrosis as a main focus for the treatment in the liver diseases.

(Yuan et al. 2019), The role of the gut microbiota in cholangiocarcinoma formation and progression is studied in this work. The authors explore how metabolic reprogramming, immunological responses and hepatic inflammation are affected by microbial dysbiosis. To prevent and treat bile duct tumors, they probe possible microbiome- targeting treatments like fecal microbiota transplantation and probiotics. This study highlights the growing connection between the risk of the liver cancer and intestinal health.

(Koo et al. 2015),The Hippo signaling pathways function in cholangiocarcinoma and lives cancer is thoroughly reviewed in this work. It indicates how deregulation of the Hippo pathway especially via YAP and TAZ activation results in unsatisfied cell growth and tumor development. They draw attention to the latest developments targeting through this pathway for cholangiocarcinoma treatment. The paper highlights new therapeutic medicines ,the possibilities of Hippo pathway inhibitors.

(Pellegrini et al. 2017), The effects of epigenetics changes in cholangiocarcinoma development are the main highlights of this work. The authors move into how non-coding RNAs, histone changes, and DNA methylation help to cause tumors. As possible treatments, they underline newly developing epigenetic medicines including histone deacetylase inhibitors and DNA methyltransferase inhibitors. The results suggest that focusing on epigenetic dysregulation, in cholangiocarcinoma, could improve the chances of patient survival.

(Tao et al. 2020), This work examines the effect of oxidative stress on CCA development. The writers investigate reactive oxygen species (RSO) as contributors to DNA damage, inflammation, and the progression of tumors. They highlight possible antioxidant-based treatments meant to reduce oxidative stress and slow down tumor development. The results suggest that a fresh strategy for cholangiocarcinoma treatment could be aimed at oxidative stress.

(Sacke et al. 2019), This work highlights the function of immunological checkpoints as therapeutic targets in cholangiocarcinoma. It indicates how PD-1,PDL-1 and CTLA-4 help to cause immune evasion in bile duct cancer. They go over clinical studies looking at immune checkpoint inhibitors like nivolumab and pembrolizumab in treating cholangiocarcinoma.

(Petersen et al . 2020), focusing on cholangiocarcinoma and liver malignancies which investigate the function of metabolic reprogramming in cancer progression, the authors explain how altered lipid metabolism, glucose metabolism and mitochondrial activity support tumor growth and treatment resistance. They go over possible metabolic targets for novel cancer treatments including medicines with mitochondrial targeting and glycolysis inhibitors. The paper emphasizes how increasingly crucial cancer metabolism is for the development of medications.

(Newton and Manning 2016), The interaction between several cell death pathways including death from apoptosis, necroptosis, and autophagy is systematically in this work. The writers go over the molecular controllers including caspases, RIPK1, and Beclin-1 that combine several pathways. They underline how knowing these connections can help to discover important weaknesses in tumor cells, therefore enhancing cancer therapy. The paper emphasizes the possibility of combination treatments aiming at several cell-killing processes.

(Kim et al. 2020), The purpose of this work

is to examine how necroptosis influences treatment resistance and cancer development. Comparing on the type of cancer and micro environment, the writers highlight that the necroptosis-mediated by RIPK1, RIPK3 and MLKL -can either repress or encourage tumor growth. They go over how necroptotic cell death could set off immunological reactions that boost antitumor immunity but might also fuel carcinogenesis, caused by inflammation. The results imply that changing necroptosis pathways could be a possible approach to cancer treatment.

(Kwak et al. 2018) explore the role of ferroptosis, an iron-dependent form of cell death, in cancer biology. Emphasizing its reliance on lipid peroxidation and iron metabolism, the authors show how ferroptosis differs from death and necroptosis. They go over possible therapeutic uses, including selectively killing cancer cells with drugs causing ferroptosis. The results underline the growing curiosity about ferroptosis as a fresh target for cancer treatment.

(Vandenabeele et al. 2010), work shows a basic knowledge of necroptosis and its control under important molecular players including MLKL, RIPK1, and RIPK3. The writers go over how necroptosis might affect inflammatory diseases, cancer, neurodegeneration, and other conditions. They draw attention to how well necroptosis inhibitors might be used as therapeutic medicines to treat pathology. The paper emphasizes the need for more investigation on the modulation of necroptosis.

(Tang et al. 2019), The several forms of programmed cell death are given a thorough review in this work together with their consequences for cancer treatment. Emphasizing the molecular controllers of each route, the writers go over death, necroptosis, ferroptosis, and autophagy. They draw attention to how changing these pathways can improve the effectiveness of cancer treatment. The study implies that combining cell death-targeting treatments could enhance therapy outcomes.

(Liu et al. 2021), This work explores the molecular pathways via which RIPK1 controls fibrosis and liver inflammation. It demonstrates that inflammatory signaling and RIPK1-mediated necroptosis help to aggravate chronic liver disease. They talk about how specifically targeted RIPK1 with particular inhibitors might help liver fibrosis and cirrhosis. The work shows a understanding of the part necroptosis plays in liver pathology.

3. MATERIALS AND METHODS

The tools and databases used in this study are AutoDock, PDB, Open Babel, UCSF Chimera, Discovery Studio, and Molsoft. They were selected from different research papers, and the structure of ligands was retrieved from the PubChem database.

A. Preparing the protein structure

The 3D structure of selected molecule RIPK1 (PDB ID: 4NEU) was acquired from the Protein Data Bank or PDB database. The three-dimensional structure of the protein is then cleaned by removing all the heteroatoms. (Figure 2)

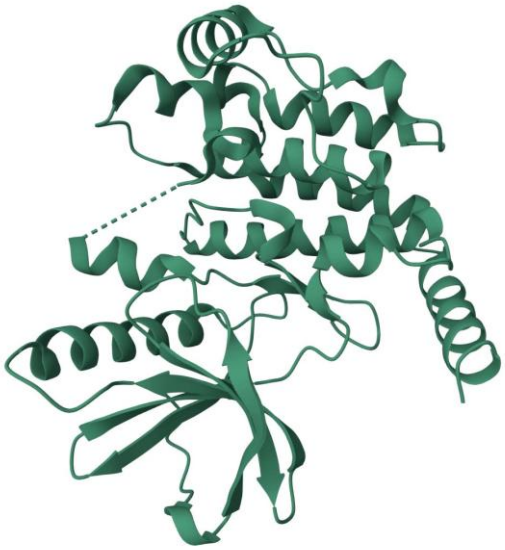

Figure 2X-ray structure of Receptor Interacting Protein 1 (RIP1) kinase domain (PDB ID: 4NEU)

Table 1-List of ligands

S.No	Ligand	Molecular Formula
1	Withanolide	C28H38O6

2	**Ginkgolide**	**C20H24O9**
3	**Silymarin**	**C25H22O10**
4	**Quercetin**	**C21H20O11**
5	**Curcumin**	**C21H20O6**
6	**Lignans**	**C22H22O8**
7	**Artemisin**	**C15H22O5**
8	**Berberine**	**C20H18NO4**
9	**Andrographolide**	**C20H30O5**
10	**Inulin**	**C12H22O11**
11	**Damnacanthal**	**C16H10O5**
12	**Nimbin**	**C30H36O9**
13	**Resveratrol**	**C14H12O3**
14	**Anthraquinone**	**C14H8O2**
15	**Hyperforin**	**C35H52O4**
16	**Forskolin**	**C12H14O2**
17	**Eugenol**	**C10H12O2**
18	**Cinnamaldehyde**	**C9H8O**
19	**Linalool**	**C10H18O**
20	**Allicin**	**C6H10OS2**

B. *Preparation of ligand structures*

The structures of the 20 antiviral drugs were referred from PubChem. The ligand structures referred from PubChem are then drawn using. The ligand structures drawn in ChemSketch is in MDL Molfiles format. This file format is not supported by the Autdock. In order to convert MDL '.mol' files to '.pdbqt' format, Open Babel software was used.

C. ***Molecular*** *Docking*

Autodock 1.5.6 was used for molecular docking. Firstly, the file path was set. The PDB file of our protein 4NEU was added

into Autodock4. The unwanted molecules other than the amino acids were removed. The missing atoms were repaired, and hydrogen was added. The Kollman charges were added. After preparing the protein molecule, our ligand, and antiviral drug is added into the Autodock. The torsion number of ligands was detected and noted.

Precalculated grid maps are required for the docking process. The grid should be around the area of interest; in this we have done blind docking. This grid coordinates covered our protein. The Lamarckian genetic algorithm (lga) was used to find the best conformers. Our 20 antiviral drugs were docked with 4NEU molecules. The '.dlg' files of docked structures at different runs with different binding energies (B.E) were produced. From these files, the conformer with the least binding energy was selected. Their root-mean-square deviation (RMSD) values were obtained. UCSF Chimera was used to analyze the hydrogen bon-based interactions in the 3D structure of the conformer.

D. *Drug likeness analysis*

Molsoft software was used for the molecular properties and drug-likeness studies of the selected ligands.

E. *ADME study*

Adsorption, Distribution, Metabolism, and Excretion (ADME) are the properties of an ideal drug. ADME studies will help to distinguish between drug and non-drug molecules. SwissADME was used for the ADME studies of the selected ligands. (Table 1)

4. RESULTS

Molecular docking studies were conducted to assess the binding strength of 20 natural plant-based ligands with RIPK1, a crucial target involved in liver cancer. The docking analysis revealed that several ligands demonstrated strong interactions with RIPK1, with Withanolide showing the highest binding affinity (-14.87 kcal/mol), followed closely by Ginkgolide (-14.83 kcal/mol) and Silymarin (-13.54 kcal/mol).

Key interactions between the ligands and RIPK1 were visualized using UCSF Chimera, showing hydrogen bonding and hydrophobic interactions with crucial amino acid residues in the active site. The drug-likeness evaluation using Molsoft indicated that the top-binding ligands adhered well to pharmacokinetic criteria, supporting their potential as lead molecules.

The ADME analysis using SwissADME further confirmed that most of the selected ligands exhibited favorable ADME profiles, with high gastrointestinal absorption and compliance with Lipinski's Rule of Five. However, some ligands displayed limited blood-brain barrier permeability, indicating potential specificity for hepatic applications.

Overall, these findings highlight Withanolide, Ginkgolide, Silymarin, Quercetin, and Curcumin as promising candidates for further experimental validation as potential therapeutic agents against hepatic cancer. (Table 2) (Figure 3-17)

Table 2-The minimum binding energy for each ligand and the corresponding docking run

S. No	Ligand	Binding energy	Run
1	Withanolide	-14.87	32
2	Ginkgolide	-14.83	12
3	Silymarin	-13.54	20
4	Quercetin	-12.11	27
5	Curcumin	-11.92	35
6	Lignans	-11.63	33
7	Artemisinin	-11.23	3
8	Berberine	-11.23	14
9	Antrographolide	-10.66	48
10	Inulin	-10.63	18
11	Damnacanthal	-9.66	13
12	Nimbin	-9.09	3
13	Resveratrol	-8.67	39

14	Anthraquinone	-8.30	49
15	Hyperforin	-8.24	39
16	Forskolin	-7.59	15
17	Eugenol	-6.80	2
18	Cinnamaldehyde	-6.07	34
19	Linalool	-5.90	33
20	Allicin	-4.96	46

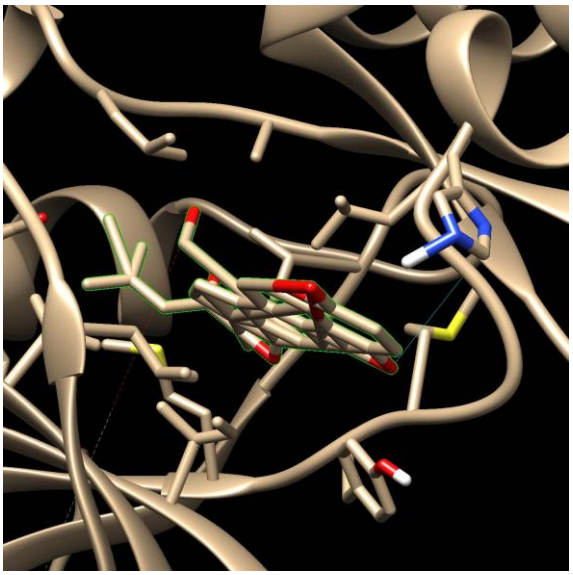

Figure 3-3D Image showing the binding of Withanolide with 4NEU

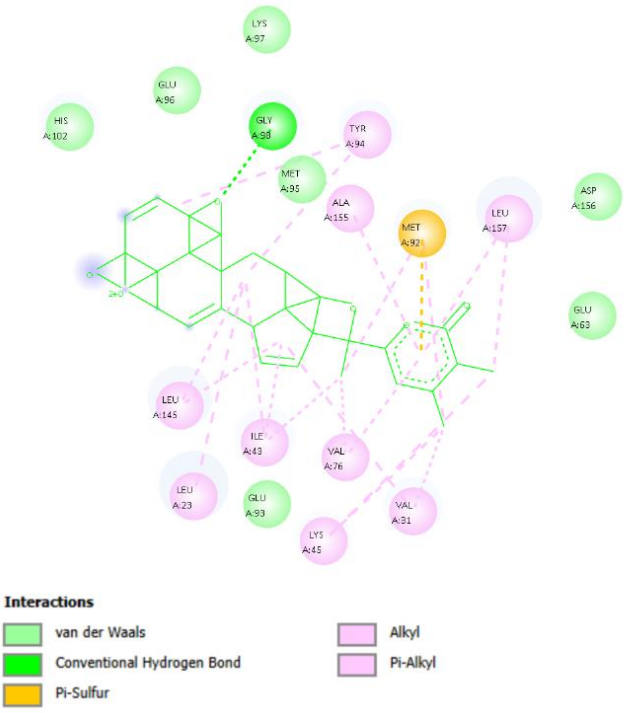

Figure 4-2D Image showing the binding of Withanolide with 4NEU

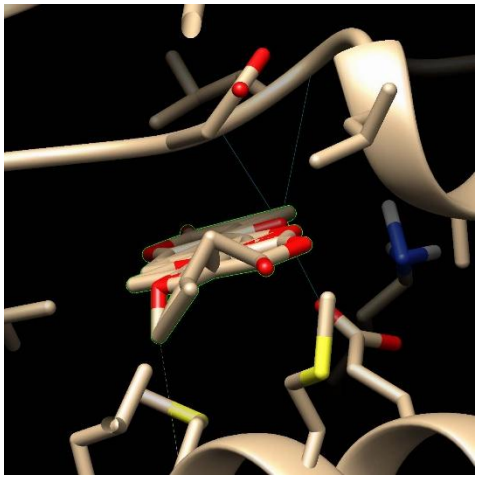

Figure 5-3D Image showing the binding of Ginkgolide with 4NEU

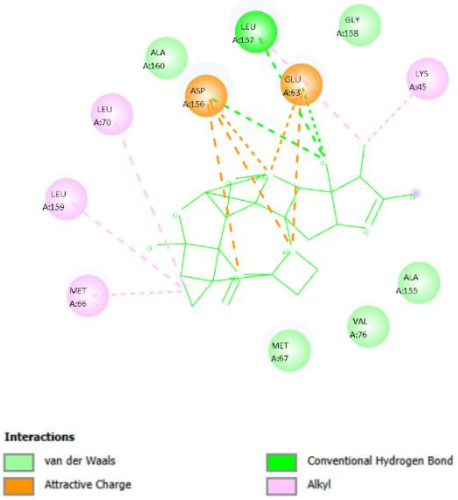

Figure 6-2D Image showing the binding of Ginkgolide with 4NEU

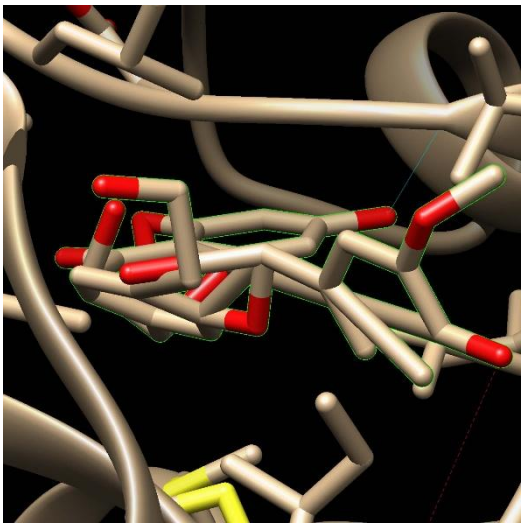

Figure 7-3D Image showing the binding of Silymarin with 4NEU

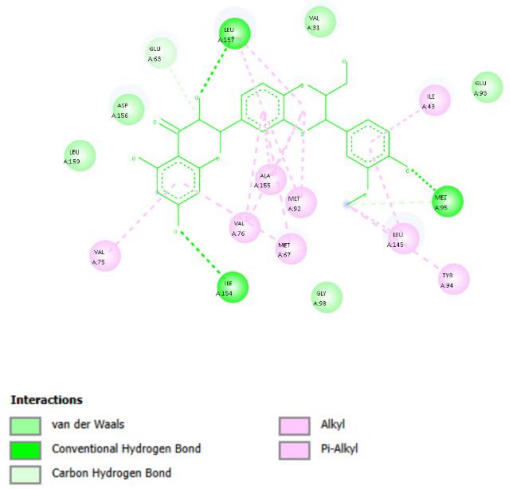

Figure 8-2D Image showing the binding of Silymarin with 4NEU

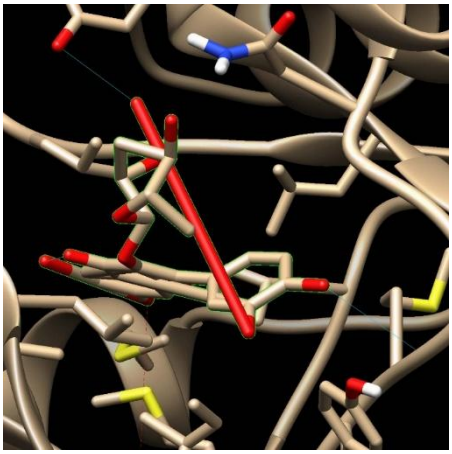

Figure 9-3D Image showing the binding of Quercetin with 4NEU

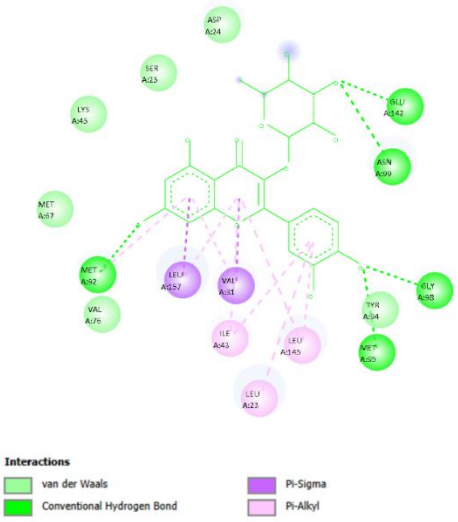

Figure 10-2D Image showing the binding of Quercetin with 4NEU

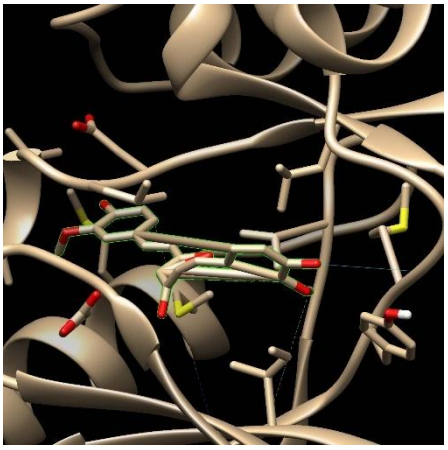

Figure 11-3D Image showing the binding of Curcumin with 4NEU

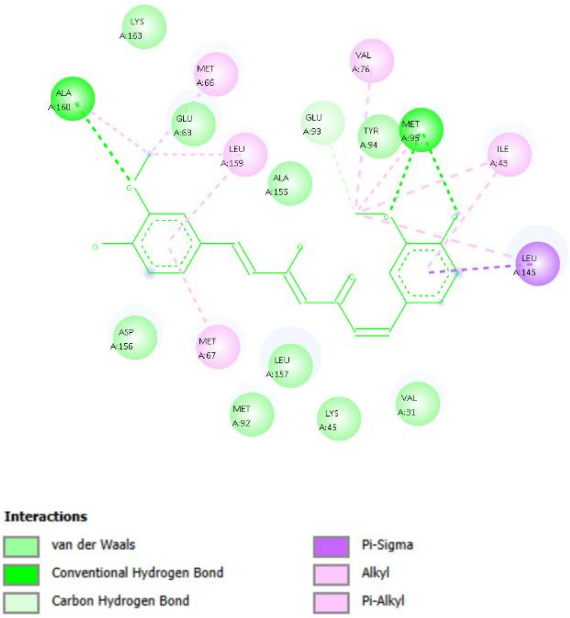

Figure 12-2D Image showing the binding of Curcumin with 4NEU

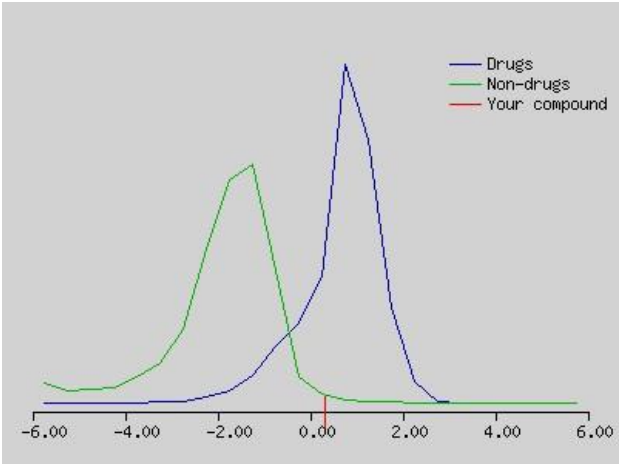

Figure 13-Drug likeness score of Withanolide

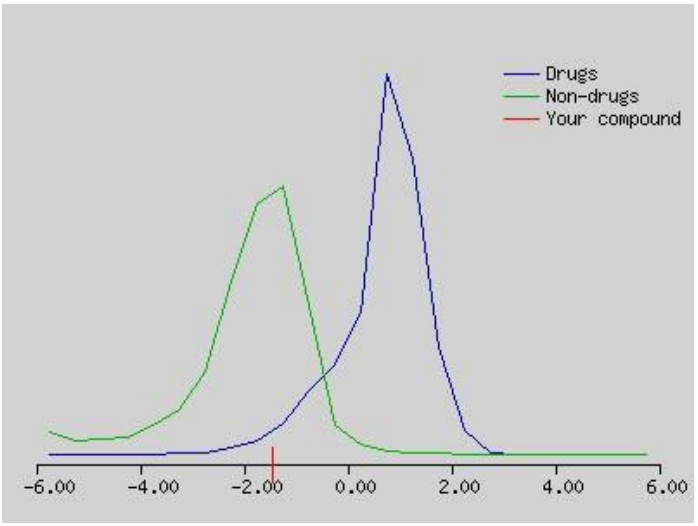

Figure 14-Drug likeness score of Ginkgolide

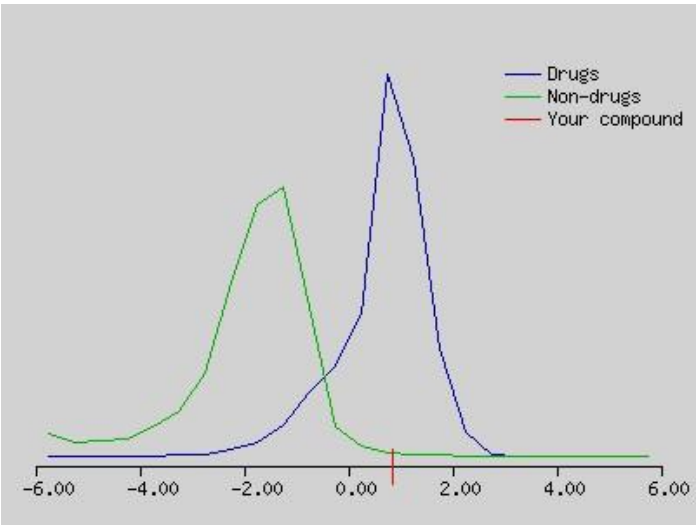

Figure 15-Drug likeness score of Silymarin

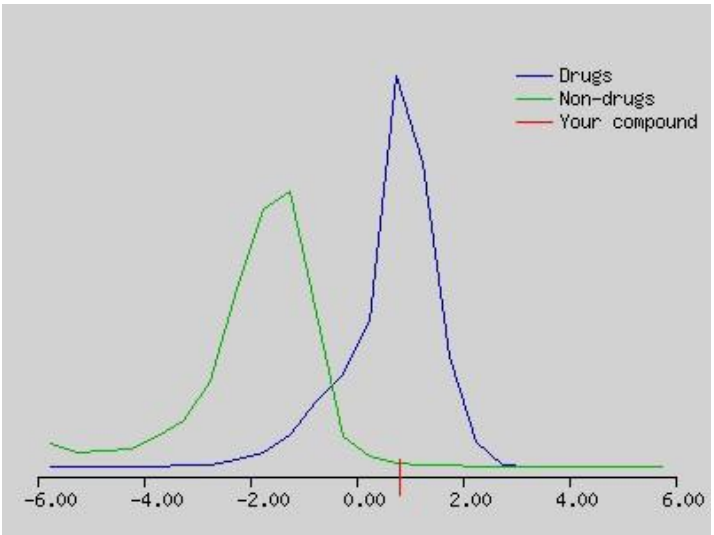

Figure 16-Drug likeness score of Quercetin

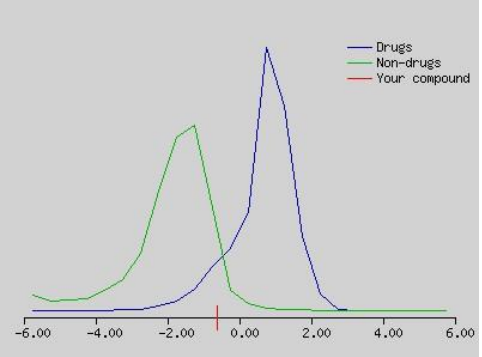

Figure 17-Drug likeness of Curcumin*Lipinski's rule of five*

(Table 3) Based on Lipinski's Rule of Five, Withanolide, Ginkgolide, Silymarin, and Curcumin meet all the criteria for good oral bioavailability, making them the most suitable candidates for drug development. In contrast, Quercetin violate two rules, suggesting lower solubility or permeability, which may affect their effectiveness as orally administered drugs.

Table 3-Table showing Lipinski's rule of five.

Ligand	Molecular weight (g/mol)	No. of HBA	No. of HBD	Mol LogP	Mol LogS	Mol PSA	Mol Vol
Withanolide	470.60	6	2	3.56	-4.02	73.94	569.93
Ginkgolide	408.14	9	2	1.02	-0.9	105.31	439.64
Silymarin	482.12	10	5	1.91	-2.24	126.94	439.51
Quercetin	448.10	11	7	0.32	-1.80	15.41	407.46
Curcumin	368.13	6	3	3.29	-2.99	77.11	401.76

A. ADME properties

(Table 4) Based on the ADME properties, Withanolide, Ginkgolide, and Curcumin show high gastrointestinal absorption (GIA) and good bioavailability (≥0.55), making them strong candidates for oral administration. Silymarin and Quercetin have low GIA, with Quercetin also showing poor bioavailability (0.17) and a pain score of 1, which may indicate potential irritation. None of the ligands cross the blood-brain barrier (BBB), suggesting limited central nervous system effects. Overall, Withanolide, Ginkgolide, and Curcumin are the most favorable in terms of ADME properties.

Table 4-Table showing ADME properties.

Ligand	BBB	Bioavailability	GIA	Skin permeability	Pain
Withanolide	No	0.55	High	-6.96	0
Ginkgolide	No	0.55	High	-8.37	0
Silymarin	No	0.55	Low	-7.89	0
Quercetin	No	0.17	Low	-8.42	1
Curcumin	No	0.56	High	-5.72	0

B. Docking select ligands to the TNF Molecule: Exploring Binding Interactions

Withanolide was successfully docked to the Tumor Necrosis Factor (TNF) molecule, yielding a binding energy of -16.22 kcal/mol in the 37th run. This strong binding affinity suggests a high potential for TNF inhibition, which is significant given TNF's role in inflammatory and autoimmune diseases. These interactions provide a strong inhibitory potential ,showing that these phytochemicals may disrupt RIPK1-mediated signaling involved in hepatic carcinogenesis .

Withanolide was successfully docked to the Tumor Necrosis Factor (TNF) molecules, yielding a binding energy of -16.22 kcal/mol at the 37th run. This strong binding affinity provides a high potential for TNF inhibition, which is significant given TNFs role in inflammatory and autoimmune diseases. Therefore, negative binding energy indicates a stable interaction between Withanolide and TNF, highlighting its assurance as a potential therapeutic candidate for targeting TNF related pathways. Ginkgolide was docked to TNF, yielding a binding energy of -14.50kcal/mol. This interaction recommends stable binding and the potential for TNF inhibition, binding and the potential for TNF inhibition, positioning it as a promising candidate for further studies in anti-inflammatory research. Silymarin demonstrated a binding energy of -13.80 kcal/mol when they docked with TNF. Their stable interaction with TNF recommends potential therapeutic applications particularly in reducing TNF-mediated inflammatory responses. Quercertin was docked to TNF, and a binding energy of -14.17kcal/mol was obtained. This strong binding interaction indicates Quercetin's potential as a TNF inhibitor. While lower than other ligands, this interaction still suggests potential TNF modulation capabilities that warrant further investigation. (Table 5) (Figure 18-28)

Table 5-The minimum binding energy for selected ligand with TNF

S. No	Ligand	Binding energy
1	Withanolide	-16.22
2	Ginkgolide	-14.50
3	Silymarin	-13.80
4	Quercetin	-14.17
5	Curcumin	-11.07

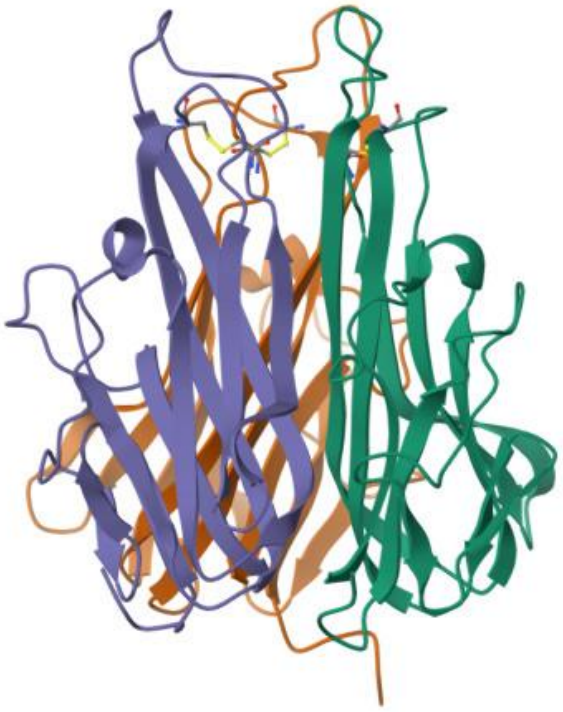

Figure 18-X-ray structure of Tumor Necrosis Factor (TNF) (PDB ID: 1TNF)

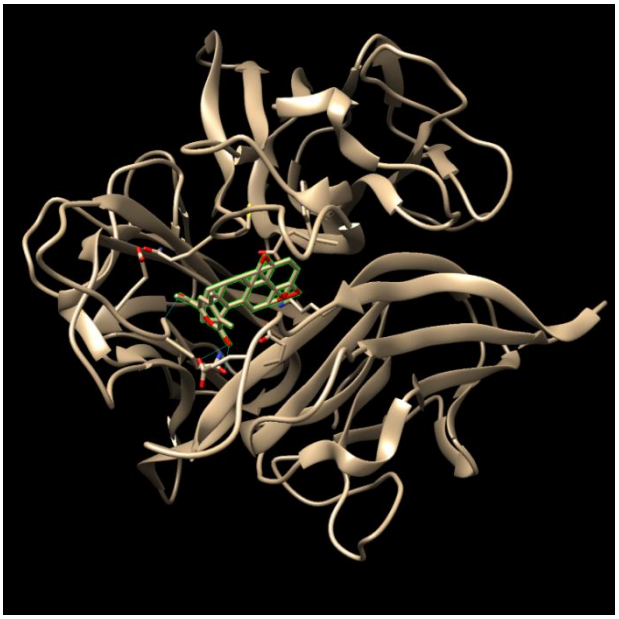

Figure 19-3D Image showing the binding of Withanolide with 1TNF

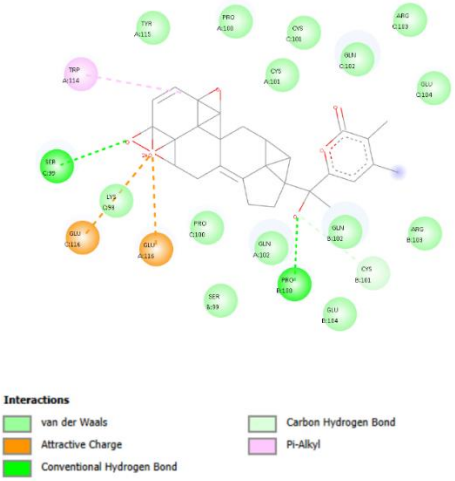

Figure 20-2D Image showing the binding of Withanolide with 1TNF

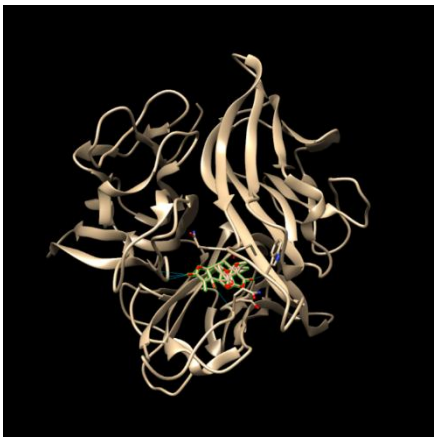

Figure 21-3D Image showing the binding of Ginkgolide with 1TNF

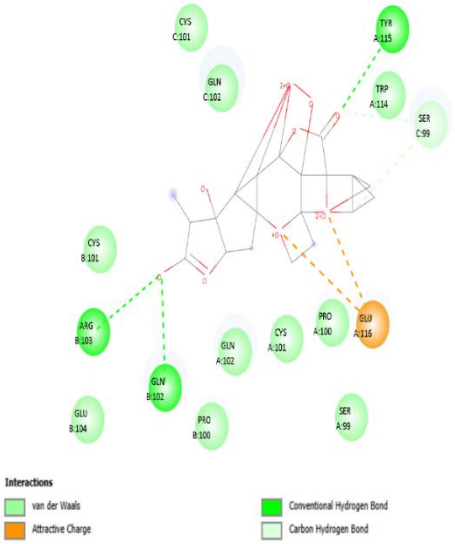

Figure 22-2D Image showing the binding of Ginkgolide with 1TNF

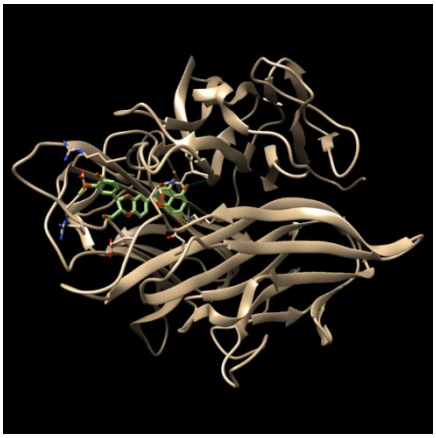

Figure 23-3D Image showing the binding of Silymarin with 1TNF

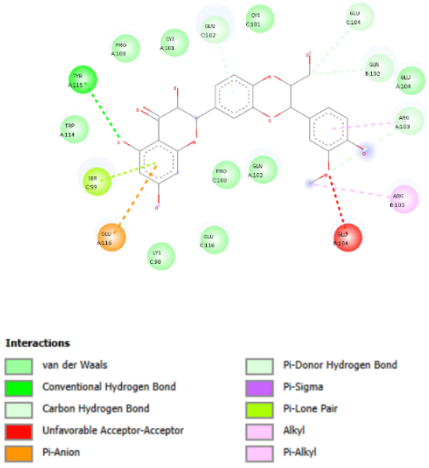

Figure 24-2D Image showing the binding of Silymarin with 1TNF

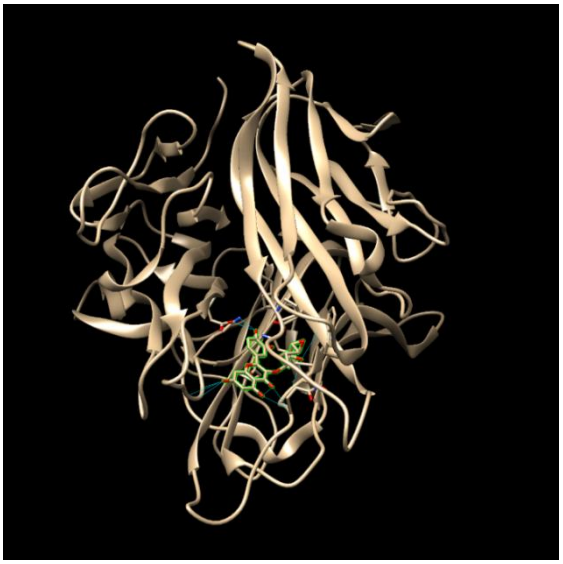

Figure 25-3D Image showing the binding of Quercetin with 1TNF

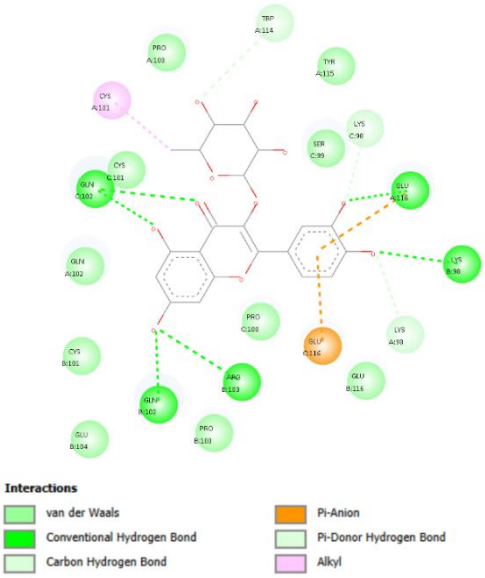

Figure 26-2D Image showing the binding of Quercetin with 1TNF

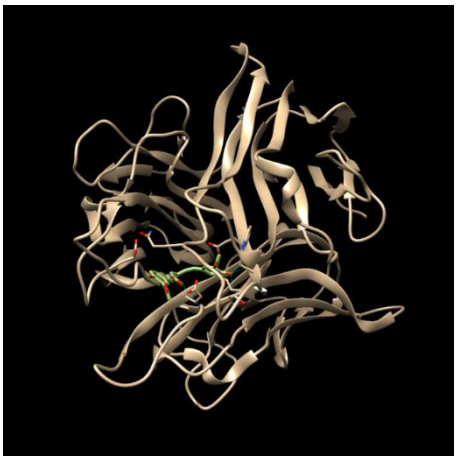

Figure 27-3D Image showing the binding of Curcumin with 1TNF

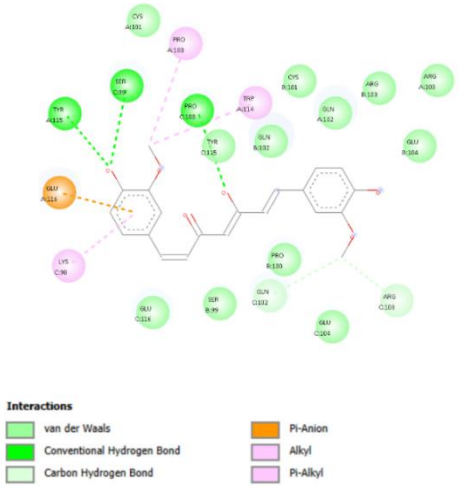

Figure 28-2D Image showing the binding of Curcumin with 1TNF

5. DISCUSSION

This study used molecular docking to investigate the potential of naturally sourced phytochemicals as inhibitors of Receptor-Interacting Protein Kinase 1 (RIPK1), a critical mediator in the progression of liver cancer, and Tumor Necrosis Factor (TNF), the protein that activates the necroptosis pathway. Given the complex etiology of cholangiocarcinoma, characterized by multiple signaling pathways and regulatory networks, the identification of phytochemicals capable of effectively targeting critical proteins represents a important step toward developing alternative therapeutic strategies.

Out of the 20 ligands analyzed, Withanolide exhibited the highest binding affinity with RIPK1 (-14.87 kcal/mol), followed closely by Ginkgolide (-14.83 kcal/mol) and Silymarin (-13.54 kcal/mol). Docking analyses revealed significant molecular interactions—including hydrogen bonding and hydrophobic contacts—between these top ligands and essential amino acid residues within RIPK1's active site. These interactions suggest a strong inhibitory potential, indicating that these phytochemicals may disrupt RIPK1-mediated signaling involved in cholangiocarcinogenesis.

Withanolide, Ginkgolide, Silymarin, Quercetin, and Curcumin, were docked to Tumor Necrosis Factor (TNF) molecule. A binding energy of -16.22 kcal/mol, -14.50 kcal/mol, -13.80 kcal/mol, -14.17 kcal/mol, -11.07 kcal/mol were obtained, respectfully, for the above-mentioned phytochemicals. These results indicate potential for TNF inhibition. The negative binging energy indicates stable interaction between the phytochemicals and the TNF molecule. Among these Withanolide has the best stable interaction. This strong interaction indicates Withanolide's potential as a TNF inhibitor, which may have implications in inflammatory disease treatment.

Drug-likeness evaluations showed that most of the selected compounds complied with Lipinski's Rule of Five, suggesting they have favorable pharmacokinetic characteristics. ADME predictions further demonstrated high gastrointestinal absorption for several candidates, supporting their potential oral bioavailability. Notably, compounds such as Quercetin and Silymarin exhibited limited blood-brain barrier permeability, implying a higher specificity for hepatic targets with reduced central nervous system exposure.

These findings are consistent with existing literature highlighting the anticancer activities of phytochemicals. For example, Curcumin has been extensively studied for its ability to modulate multiple oncogenic pathways, while Silymarin is recognized for both hepatoprotective and anticancer properties. The high binding affinities observed in this study reinforce the therapeutic potential of these natural compounds.

Nonetheless, molecular docking provides a theoretical prediction based on structural compatibility and does not confirm biological efficacy. Thus, experimental validation using in vitro cytotoxicity assays and in vivo animal models is crucial to confirm these findings. Future studies should assess the ability of these phytochemicals to induce apoptosis and inhibit proliferation in hepatic cancer cell lines, as well as evaluate their pharmacodynamic profiles and potential toxicity in animal models.

Moreover, evaluating integrative effects with the standard chemotherapeutic agents would reveals collective interactions, potentially enhancing therapeutic efficacy while minimizing adverse effects. These strategies could support in the development of more effective and less toxic treatment possibilities for liver cancer.

6. CONCLUSION

This study highlights the therapeutic potential of naturally derived phytochemicals—Withanolide, Ginkgolide, Silymarin, Quercetin, and Curcumin—as dual inhibitors of two critical proteins: RIPK1, a key player in liver cancer progression, and Tumor Necrosis Factor (TNF), a major driver of inflammation and immune responses. These compounds not only showed strong binding affinity to RIPK1 but also demonstrated stable interactions with TNF, specifying that they could serve a dual role in targeting both the cancerous and inflammatory pathways.

Among all the compounds analyzed, Withanolide stood out as the most promising candidate. Withanolide shows the highest binding affinity for the both RIPK1(-14.87 kcal/mol) and TNF (-16.22 kcal/mol), by providing a strong and stable interaction at the molecular level. This binary targeting ability is mainly significant because inflammation and cancer are often inter-linked chronic inflammation can contribute to cancer development and progression. Hence compounds like Withanolide which can be potentially address both the aspects may hold greater therapeutic value.

The docking results are further carried up by favorable pharmacokinetics predictions, including high gastrointestinal absorption and good concession with drug-likeness criteria such as Lipinski's Rule of Five . These findings provides that the selected phytochemicals have the characteristics sensible for further drug development, particularly for vocal administration.

Moreover, it is the important to remember that the molecular docking is just the first step ,which is a virtual simulation that predicts how the molecules might interact. While this offers a valuable direction, the real-world effectiveness of these compounds which must be verified through the laboratory experiments. In vitro studies will be needed to determine how these phytochemicals affect hepatic cancer cells, whether they induce cell death, and how selectively they act on cancerous versus healthy cells. In vivo studies, using animal models, will help assess safety, toxicity, and how the compounds behave in a living system.

In conclusion, this study lays a solid groundwork for further investigation of plant-based compounds in cancer treatment. The ability of compounds like Withanolide to target both RIPK1 and TNF highlights a promising strategy for developing more effective and multi-functional therapeutics. Integrating computational tools with experimental research can greatly accelerate the drug discovery process—saving time, reducing costs, and guiding attention toward the most promising candidates. With continued research and validation, these phytochemicals could one day lead to new, natural treatment options for patients battling hepatic cancer and inflammation-driven diseases

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