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Araştırma Makalesi / Research Article

Investigating the Potential of Natural Agents for Colorectal Cancer Treatment with Molecular Modeling

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Abstract

Colorectal cancer occurs with the uncontrolled growth of cells in the inner part of the large intestine. The most important factor playing a role in the development of colorectal cancer is mutations in the adenomatous polyposis coli (APC) gene. Irinotecan and folinic acid, which are chemical drugs used in the chemotherapy treatment of colorectal cancer, have many side effects on the human body. For this reason, solutions are being sought with natural agents as alternatives to chemical drugs, using in silico environments where less costly and faster drug design can be done day by day. In addition to the drugs mentioned, this study modeled the interaction of withaferin A, epigallocatechin gallate (EGCG), and ganoderiol A with APC using molecular docking and dynamic simulations. In docking studies, the interaction energy results of withaferin A, (EGCG), and ganoderiol A are -8.5, -7.8, and -7.9 kcal/mol, respectively. In MD simulations, the average RMSD values of these three agents are 0.5 nm for withaferin A and ganoderiol A and 1.5 nm for the more branched, bulky EGCG. The modeling results reveal the therapeutic drug potential of natural agents on the APC gene associated with colorectal cancer and provide a basis for other studies.

Keywords: APC gene, Colorectal cancer, Molecular modeling, Natural agent.

Moleküler Modelleme ile Kolorektal Kanser Tedavisinde Doğal Ajanların Potansiyelinin Araştırılması

Öz

Kolorektal kanser, kalın bağırsağın iç kısmındaki hücrelerin kontrolsüz büyümesiyle oluşur. Kolorektal kanser gelişiminde rol oynayan en önemli faktör adenomatöz polipozis koli (APC) genindeki mutasyonlardır. Kolorektal kanserin kemoterapi tedavisinde kullanılan kimyasal ilaçlardan olan irinotekan ve folinik asit insan vücudu üzerinde birçok yan etkiye sahiptir. Bu nedenle kimyasal ilaçlara alternatif olarak doğal ajanlarla, her geçen gün daha az maliyetli ve daha hızlı ilaç tasarımı yapılabilen in silico ortamlarda çözümler aranmaktadır. Bahsedilen ilaçlara ek olarak, bu çalışmada withaferin A, epigallocatechin gallate (EGCG) ve ganoderiol A'nın APC ile etkileşimi moleküler yerleştirme ve dinamik simülasyonlar kullanılarak modellenmiştir. Yerleştirme çalışmalarında withaferin A, (EGCG) ve ganoderiol A'nın etkileşim enerjisi sonuçları sırasıyla -8,5, -7,8 ve -7,9 kcal/mol'dür. MD simülasyonlarında, bu üç ajanın ortalama RMSD değerleri withaferin A ve ganoderiol A için 0,5 nm ve daha dallanmış, hacimli EGCG için 1,5 nm'dir. Modelleme sonuçları, doğal ajanların kolorektal kanserle ilişkili APC geni üzerindeki terapötik ilaç potansiyelini ortaya koyabileceği ve diğer çalışmalar için bir temel sağlayacağı düşünülmektedir.

Anahtar Kelimeler: APC geni, Kolorektal kanser, Moleküler modelleme, Doğal ajan.

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1. Introduction

Colorectal cancer, which is one of the most common types of cancer, occurs when cells in the large intestine grow and multiply abnormally (Siegel, Miller, & Jemal, 2020). One of the most effective factors in the occurrence of colorectal cancer is a gene mutation (Olkinuora, Peltomaki, Aaltonen, & Rajamaki, 2021). The gene mutation that causes colorectal cancer to progress is related to the APC gene (Shailes, Tse, Freitas, Silver, & Martin, 2022). APC abnormally activates the Wnt signaling pathway, leading to the progression of colorectal cancer (Chicurel, 2002; Polakis, 2007). The APC gene is found mutated in the majority of colorectal cancers, and these mutations usually occur in the early stages of cancer (Kinzler & Vogelstein, 1996). For this reason, it is very important to find alternative methods for the treatment of APC gene-related colorectal cancer (Peng et al., 2023). As with every type of cancer, there are many methods for the treatment of colorectal cancer, such as radiotherapy and chemotherapy (Gmeiner, 2024). While these methods help treat cancer, they also have many side effects on the human body, such as nausea, collapse of the immune system, and damage to the functions of vital organs (Li, Zhang, & Xu, 2023). In addition to these methods, the use of chemical drugs also has further side effects on the human body (Bontempo, De Masi, & Rigano, 2023). For this reason, finding natural alternative medicine agents with very few side effects is very important for human health.

Natural agents such as flavonoids, steroidal lactones, alkaloids, saponins and triterpenoids contain many bioactive substances such as antioxidants, anticancer and anti-inflammatory agents thanks to their different chemical structures. (Dar, Shahnawaz, Ahanger, & Majid, 2023). With these bioactive properties, they show therapeutic effects for many diseases, especially colorectal cancer. Natural agents have become very popular in recent years as they are used in the treatment of many diseases as an alternative to chemical drugs. The reason for this is that they have almost no side effects compared to chemical drugs (Chaachouay & Zidane, 2024). In addition, the fact that a natural agent has many bioactive properties such as antioxidant and anticancer effects offers a versatile treatment strategy for disease management. (Guru, Kar, Nayak, & Mohapatra, 2023).

The main reason for choosing natural agents Withaferin A, EGCG, and Ganoderiol A as ligands in this study is that they have mechanisms suitable for reducing the side effects of traditional chemotherapy and modulating damaged signaling pathways that cause cancer progression (Choi & Kim, 2015; Gao, Hirakawa, Min, Nakamura, & Hattori, 2005; Nasir et al., 2020). In addition, the chemical structure of each ligand is predicted to interact well with the APC gene, which is associated with colorectal cancer, through a molecular modeling approach. For example, withaferin A's steroidal lactone structure, EGCG's polyphenolic structure, and ganoderiol A's triterpenoid-bearing chemical structures are predicted to have the potential to establish strong hydrogen bonds and hydrophobic

interactions with the APC gene, and thus may be effective against colorectal cancer (Macharia, Kaposztas, & Bence, 2023; Wahnou et al., 2023). For these reasons, it establishes strong hydrogen bonds with the gene receptor, resulting in a strong interaction. The polyphenolic structure of EGCG and the triterpenoid structure of the ganoderiol A ligand have the potential to interact effectively with the APC gene by establishing hydrophobic interactions (Han et al., 2023; Macharia et al., 2023; Wahnou et al., 2023). For these reasons, these ligands were preferred for investigation within the scope of this study to examine the drug potential of natural agents, using as a reference chemical agents that have many side effects but are widely used as anticancer drugs on the market.

In this study, we aimed to reveal the anticancer properties of natural agents such as withaferin A, EGCG, and ganoderiol A, compared to chemical drugs such as folinic acid and irinotecan, by using molecular modeling approaches in the treatment of colorectal cancer associated with the APC gene using the crystal receptor structure with PDB ID 3AU3. Specifically, the binding abilities of natural agents to the APC protein were examined by molecular docking analyses, and the stability of the resulting receptor-ligand complexes was analyzed by molecular dynamics simulations. Since there is no study in the literature examining the effect of natural agents using a molecular modeling approach with the APC gene associated with colorectal cancer, this study is intended to form the basis of many future literature studies.

2. Materials and Methods

2.1. Protein Preparation

The 3D structure of the APC gene (PDB ID: 3AU3) was obtained from the RCSB Protein Data Bank (Figure 1). The protein structure was prepared using AutoDock Tools 1.5.7 by removing water molecules, adding polar hydrogen atoms, Kollman partial charges, and converting the file to the AD4 atom type .pdbqt format (G. M. Morris et al., 2009).

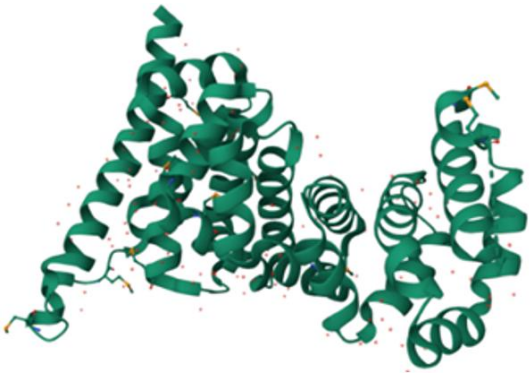

Figure 1. Crystal structure of APC protein (PDB ID: 3AU3).

2.2. Ligand Preparation

The 3D structures of several compounds were retrieved from the PubChem database and prepared for docking analysis. The natural products include withaferin A (CID:155887202), EGCG (CID: 65064), and ganoderiol A (CID: 100927467) shown in Figure 2. Additionally, two conventional drugs, irinotecan (CID: 60838) and folinic acid (CID: 135403648) shown in Figure 2 were also obtained. Using PyMOL (Molecular Graphics System, Version 2.0 Schrödinger, LLC.), the downloaded files were converted to the .pdb file format. Subsequently, Chimera Tools software was utilized to prepare the ligand files, which were finally written in .pdbqt format to facilitate the docking analysis (Pettersen et al., 2004).

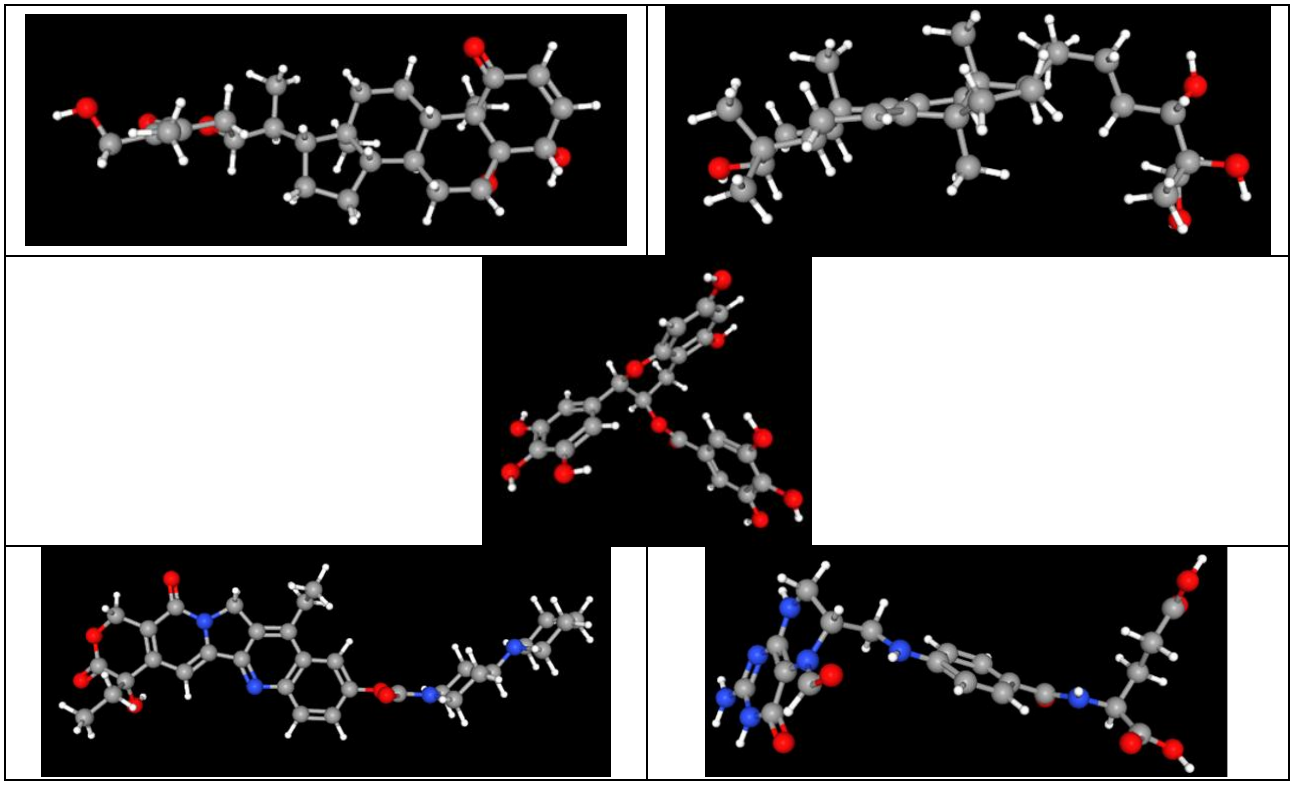

Figure 2. The 3D structure of withaferin A, epigallocatechin gallate, ganoderiol A, irinotecan, and folinic acid, respectively. The red, blue, gray, and white colors represent oxygen (O), nitrogen (N), carbon (C), and hydrogen (H).

2.3. Molecular Docking Analysis

Molecular docking analysis was performed using Chimera Tools software to dock two conventional drugs and three natural agents into the active site of the APC protein. The active site coordinates of the APC protein were determined via global alignment, and five grid boxes were created specifically for this active site. Dockings were performed with Chimera Computer-based

molecular modeling tools were used to analyze the binding interactions of each ligand. The coordinates of the active site center of the receptor and the grid box dimensions are shown in Table 1. As a result of the analysis, the aim was to determine the score of the lowest binding energy and estimate the inhibition constant. This way, information was provided regarding the potential interactions between the ligands and the APC protein selected as the receptor (Qawoogha & Shahiwala, 2020).

Table 1. Grid box sizes and center coordinates used in molecular docking studies.

center of active site	grid box dimensions (x,y,z)
x= -52.734 y= 36.466 z= 5.797	20 x 20 x 20
	40 x 40 x 40
	50 x 50 x 50
	60 x 60 x 60
	70 x 70 x 70

2.4. Protein-Ligand Complex Visualization

The protein-ligand complexes obtained from the analyses were visualized with Discovery Studio Visualizer 20.1 software (Qawoogha & Shahiwala, 2020; Zhang, Cheng, Jin, Zhao, & Wang, 2021). Thanks to this software, polar and hydrophobic interactions between the ligand and target were characterized and protein-ligand complex map representations of the interactions were created.

2.5. Utilization of SwissADME for Prediction of Pharmacokinetic Properties

The physicochemical properties and potential drug-like characteristics of ligands were calculated using SwissADME. SwissADME provides comprehensive predictions including pharmacokinetics, drug-likeness, and medicinal chemistry friendliness (Daina, Michielin, & Zoete, 2017).

2.6. Molecular Dynamics Simulation

A 50 ns molecular dynamics simulation was performed to analyze the properties such as stability and compactness of the complexes obtained after docking the APC protein, selected as the receptor, with five different ligands. RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), RDF (Radial Distribution Function), and Rg (Radius of Gyration) graphs were

obtained using the GROMACS program for the simulation. Thanks to these graphs, both physical and thermodynamic properties such as conformational changes, stability, compactness, and density between the protein and ligands were analyzed (Yadav et al., 2022; Zrinej, Elmchichi, Alaqarbeh, Lakhlifi, & Bouachrine, 2023).

3. Findings and Discussion

When the results were examined in general within the scope of this study, among traditional drugs, irinotecan exhibited a strong interaction with the APC gene, with a binding energy of -8.5 kcal/mol. Additionally, another drug, folinic acid, showed a binding affinity of -8.1 kcal/mol. Among natural ligands, EGCG and Ganoderiol A showed the best interactions with the target receptor, with binding energies of -7.8 and -7.9 kcal/mol, respectively. When the comparative pharmacokinetic properties of natural agents were examined, it was observed that the molecular weights of Ganoderiol A (478.75 g/mol) and Withaferin A (472.61 g/mol) were lower than those of marketed drugs. Furthermore, it was noted that natural agents Withaferin A and Ganoderiol A demonstrated good oral bioavailability, particularly with gastrointestinal properties similar to irinotecan. When the potential of natural agents as drug candidates was examined, Withaferin A and Ganoderiol A were found to meet all established criteria and were considered candidate drugs according to Lipinski's rule. In molecular dynamics simulation studies, RMSD, RMSF, RDF, and Rg graphs of receptor-ligand complexes were obtained. All ligands generally exhibited RMSD values between 0.75 and 1.75 nm on the APC gene receptor. However, the natural agent Ganoderiol A showed the most stable interaction with the receptor. In the RMSF graphs, the ligand that exhibited the least flexibility was the EGCG ligand, with a value of 0.2 nm. In the RDF graphs, Ganoderiol A demonstrated the strongest interaction with the APC gene, achieving the best peak point. Rg values generally indicated that all receptor-ligand complexes had a compact structure in the findings. However, it was observed that Ganoderiol A and Withaferin A formed a much more compact complex structure, with an Rg value of approximately 2.3 nm. Additionally, the EGCG ligand exhibited a compact structure with its highly stable Rg value.

3.1. Binding Energy and Hydrogen Bonds

The binding affinities of various natural compounds and conventional drugs to the APC protein were evaluated through molecular docking analyses using different grid box sizes. The docking results are summarized in Table 2.

Table 2. Binding energy (kcal/mol) of conventional drugs and natural compounds to APC protein across different grid box sizes for molecular docking results.

Target Protein	Ligand	Binding Energy (kcal/mol)				
		20x20x20	40x40x40	50x50x50	60x60x60	70x70x70
APC	Irinotecan	-7.8	-7.2	-8.1	-8.5	-8.4
	Folinic acid	-7	-7.3	-7.3	-8.1	-7.3
	WithaferinA	-7.2	-7	-8.5	-8.5	-8.4
	Epigallocatechin Gallate	-5.9	-7.3	-7.5	-7.8	-7.8
	Ganoderiol A	-6.0	-7.2	-7.7	-7.9	-7.0

Table 2 provides a comprehensive overview of the binding energies of various compounds, both conventional chemotherapeutic agents and natural compounds when docked to the APC protein. The binding energy is a critical parameter as it indicates the strength and stability of the interaction between the compound and the target protein (G. M. Morris et al., 2009). Lower binding energy values correspond to stronger binding affinities, which are desirable for effective inhibition of the target protein (Zrinej et al., 2023).

The results indicate that irinotecan exhibits the highest binding affinity to the APC protein, particularly at a grid box size of 60x60x60, with a binding energy of -8.5 kcal/mol. Additionally, irinotecan formed seven hydrogen bonds with the APC protein, with bond lengths ranging from 1.85 to 2.83 Å. In addition, ARG158 has a 2.4 Å long hydrogen bond between its oxygen atom and the ligand (Figure 3).

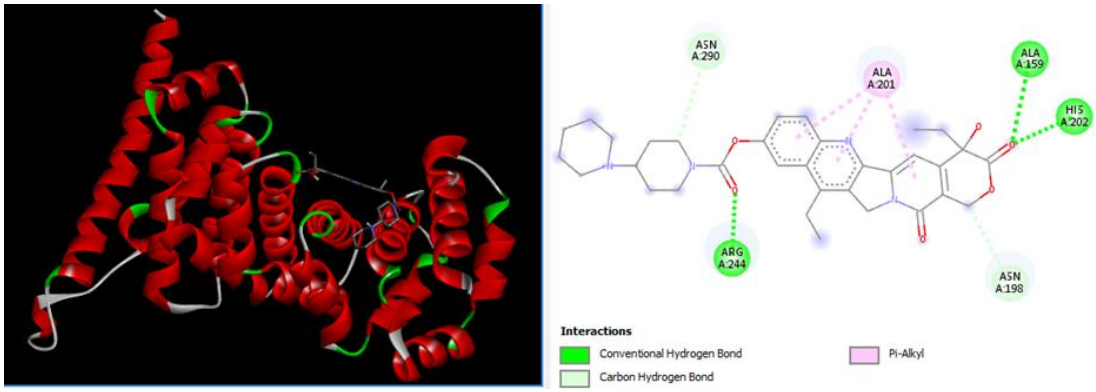

Figure 3. Irinotecan binding to APC protein, illustrating significant hydrogen bonds.

Folinic acid also demonstrated significant binding affinity, with its highest binding energy of -8.1 kcal/mol at the 60x60x60 grid box size. The interaction analysis revealed that folinic acid formed ten hydrogen bonds with the APC protein, with bond lengths ranging from 2.08 to 2.8 Å. A significant interaction was observed between the oxygen of ARG153 and folinic acid, measuring 2.24 Å (Figure 4). These findings underscore the effectiveness of irinotecan and folinic acid as conventional treatments for colorectal cancer, with strong and stable binding affinities to the APC protein.

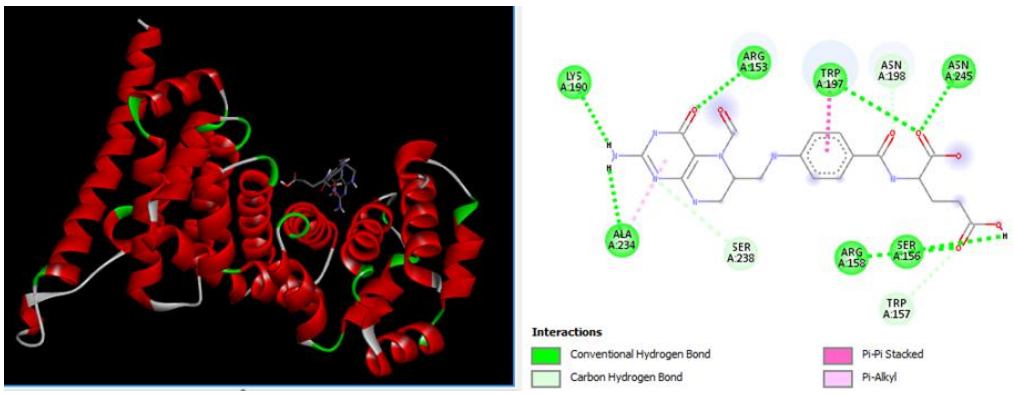

Figure 4. Folinic acid binding to APC protein, presenting crucial interactions.

Among the natural compounds, withaferin A showed the highest binding energy of -8.5 kcal/mol at the 50x50x50 grid box size, indicating a strong and stable interaction with the APC protein. Withaferin A formed six hydrogen bonds with the APC protein, with bond lengths ranging from 2.12 to 3.14 Å. An important interaction included a hydrogen bond between the oxygen of ARG153 and withaferin A, measuring 2.5 Å (Figure 5).

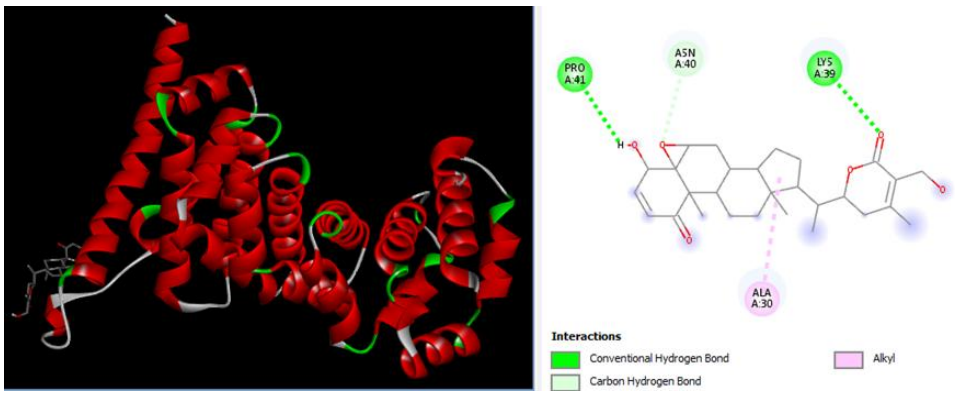

Figure 5. Withaferin A binding to APC protein, showing detailed interaction plots.

EGCG exhibited a binding energy of -7.8 kcal/mol at both the 60x60x60 and 70x70x70 grid box sizes, suggesting good binding affinity. EGCG established ten hydrogen bonds with the APC protein, with bond lengths ranging from 2.27 to 2.94 Å. A key interaction involved a hydrogen bond between the oxygen of TRP157 and EGCG, measuring 2.7 Å (Figure 6).

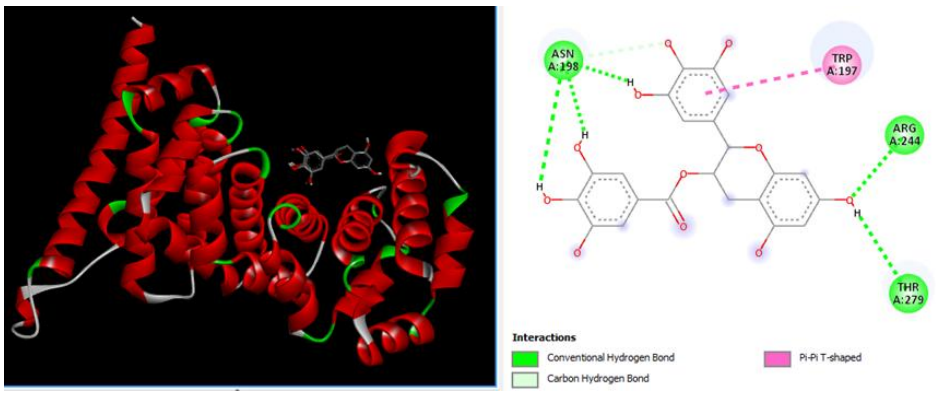

Figure 6. EGCG binding to APC protein, highlighting polar and hydrophobic interactions.

Ganoderiol A exhibited a binding energy of -7.9 kcal/mol at the 60x60x60 grid box size, which is also significant and comparable to the binding energies of conventional drugs. Ganoderiol A formed nine hydrogen bonds with the APC protein, with bond lengths ranging from 1.92 to 2.95 Å. A significant interaction was observed between the oxygen of ARG67 and the ligand, measuring 2.49 Å (Figure 7).

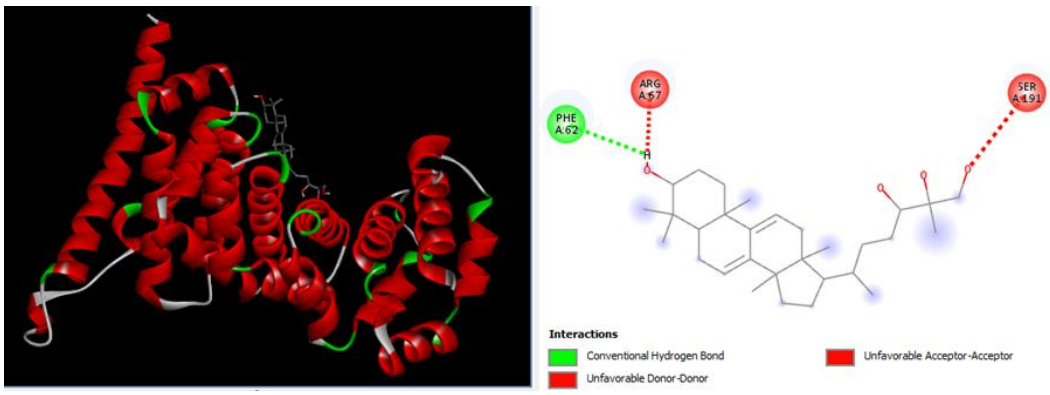

Figure 7. Ganoderiol A binding to APC protein, depicting key hydrogen bonds.

The compounds investigated include Withaferin A, EGCG, and Ganoderiol A, and we compared them to traditional chemotherapy agents such as Irinotecan and Folinic acid. The studied parameters, such as pharmacokinetics, drug similarity, water solubility, and physicochemical properties, are shown in Table 3.

Table 3. Comparative analysis of water solubility, pharmacokinetics, and druglikeness of selected compounds.

Ligand name	Water Solubility		Pharmacokinetics		Druglikeness			
	Log S (ESOL)	Log S (Ali)	GI adsorption	BBB permeant	Lipinski	Ghose	Veber	Egan
Irinotecan	-2.99	-2.25	High	No	Yes	No	Yes	Yes
Folinic acid	-0.63	-1.61	Low	No	No	No	No	No
WithaferinA	-4.98	-5.54	High	No	Yes	No	Yes	Yes
Epigallocatechin Gallate	-0.83	-0.89	Low	No	No	No	No	No
Ganoderiol A	-6.58	-8.11	High	No	Yes	No	Yes	Yes

The molecular weights of the natural compounds range from 472.61 g/mol (Withaferin A) to 478.75 g/mol (Ganoderiol A), which are slightly lower than those of the traditional drugs, particularly Irinotecan (597.77 g/mol). Water solubility, indicated by Log S (ESOL) and Log S (Ali), shows that all the natural compounds are less soluble in water compared to Folinic acid, with Ganoderiol A having the least solubility among them (Yalcin, 2020).

From a pharmacokinetic perspective, both Withaferin A and Ganoderiol A demonstrate high gastrointestinal (GI) absorption, similar to Irinotecan, suggesting good oral bioavailability potential for these natural agents. In terms of blood-brain barrier (BBB) permeation, none of the compounds, including both traditional and natural, are predicted to cross the BBB, indicating limited central nervous system side effects (El Fadili et al., 2022; Yalcin, 2020).

When assessing drug-likeness using Lipinski's rule, all compounds except Folinic acid conform to Lipinski's criteria, suggesting good oral bioavailability. Further analysis using the Ghose, Veber, and Egan filters indicates that Withaferin A and Ganoderiol A satisfy all three filters, highlighting their strong druglikeness potential. EGCG does not meet Ghose's criteria, and Folinic acid only conforms to Lipinski's rule (Moshawih et al., 2022). In summary, the analysis suggests that Withaferin A and Ganoderiol A are promising candidates for further investigation as colorectal cancer treatments. Their physicochemical properties, pharmacokinetics, and druglikeness profiles compare favorably to traditional agents, highlighting their potential as natural alternatives in therapeutic applications (El-Mernissi et al., 2024).

3.2. Molecular Dynamics Simulation Analysis

Within the scope of this study, a 50 ns molecular dynamics simulation was conducted to highlight the therapeutic effect of the ligands and their interaction with the protein. As a result of the simulation, RMSD, RMSF, RDF, and Rg graphs (Figure 8-11) were obtained.

RMSD - analyzes the mean square deviations of the changes in the positions of a protein or receptor-ligand complex structure with reference to the starting points of the atoms of a particular molecule (Akash et al., 2024). Due to RMSD graphs, the structural stability of receptor-ligand complexes, binding affinities and changing conformational properties of the complex are examined (Chen et al., 2023). When the RMSD graphs of the complexes consisting of the APC protein and the ligands EGCG folinic acid, ganoderiol A, irinotecan, and withaferin A shown in Figure 8 are examined, the fact that the RMSD values of all ligand complexes are between 0.75 and 1.75 nm indicates that generally good data are obtained. The average RMSD values of the receptor complex formed with the EGCG ligand during the 25 ns simulation are around 0.5 to 0.6 nm. These values show that the complex structure formed with the EGCG ligand and APC protein is quite stable. Sharp peaks were observed in this complex at 13 and 21 ns, but since there is not much increase in value in these peaks on a nm basis, it does not lose its stability. The RMSD graphs of the complex structures formed with folinic acid and withaferin A ligands are quite similar to each other. While the average RMSD values of the complex structure formed with folinic acid are 0.6 nm, the withaferin A complex is around 0.7 nm. It can be seen that these two different ligand complexes fluctuate from time to time throughout the simulation. This shows that some conformational changes occur while the receptor and ligands bind to each other (Akash et al., 2024; Chen et al., 2023). The complex structure formed with the APC protein and the irinotecan ligand shows a more irregular and unstable graph compared to other ligand complexes. The average RMSD values of this complex are around 1.30 nm, and higher data were obtained than the RMSD values of other complexes. The fact that the irinotecan complex graph fluctuates and peaks throughout the simulation shows that the ligand and the receptor undergo conformational changes and form an unstable complex structure (Chen et al., 2023). In addition, the complex formed with the ganoderiol A ligand shows the best results compared to all ligand complexes. The RMSD values obtained throughout the simulation range between 0.2 and 0.3 nm and have the lowest RMSD values. Its graphical appearance remained very stable, showing almost no fluctuation, especially after 0.18 ns. Among these ligands, ganoderiol A forms the best and most stable complex structure with the APC protein. This situation creates a good impression in terms of research on the ganoderiol A ligand for the treatment of colorectal cancer and its use as an alternative medicine (Ye et al., 2023).

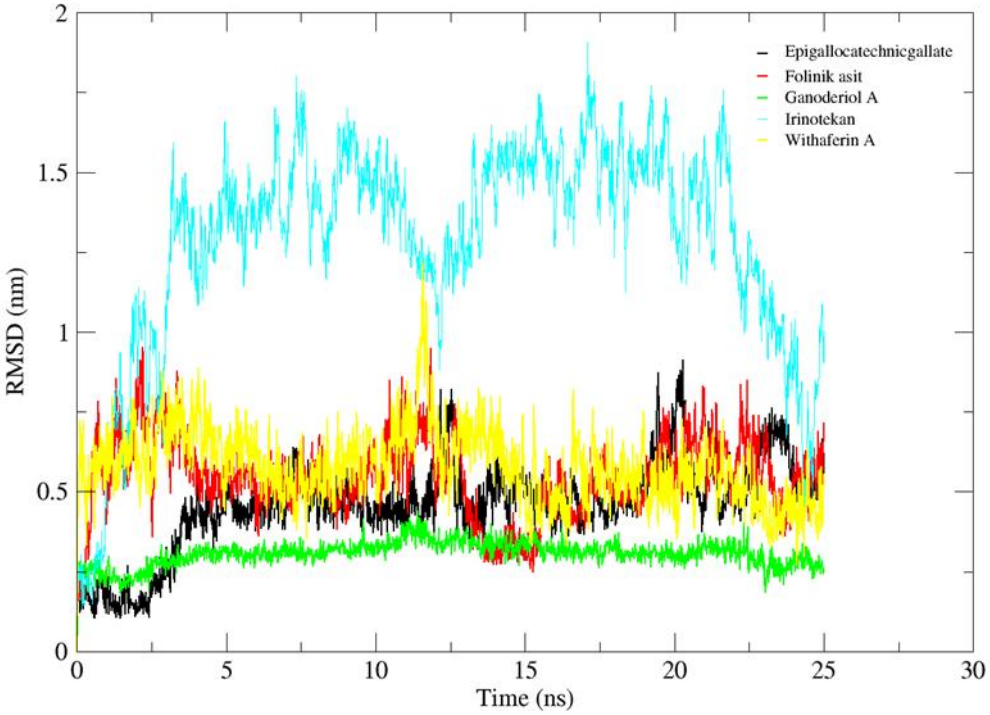

Figure 8. RMSD graph between the backbone of the receptor and ligands of epigallocatechin gallate, folinik asit, ganoderiol A, irinotekan and, withaferin A, respectively.

RMSF analyzes the movement and flexibility of atoms or molecules by measuring the deviations of their average positions relative to the reference structure (Akash et al., 2024). Within the scope of molecular dynamics simulation, RMSF graphs are used to examine the flexibility of the protein and ligand complex, the conformational changes that occur in the receptor, ligand, and binding region during ligand binding, and to examine the effects of mutations in the structure, if any (Chen et al., 2023). When the RMSF graphs of the APC receptor and five different ligand complexes in Figure 9 are examined, generally similar graphs are seen. RMSF values of the EGCG complex are generally below 0.2 nm. This shows that the EGCG ligand binds to the receptor in a compact manner and is quite stable. In general, all ligand complexes showed graph values extending to 0.6 nm at 800 to 1000 atoms, as in the case of the EGCG ligand. The reason for this difference can be interpreted as this region of the receptor having a dynamic function (Skariyachan et al., 2020). When the RMSF graphs of other ligand complexes formed with the APC receptor are examined, the average RMSF values of all complexes are 0.2 nm and below. The fact that the values of these ligands are so low and there is not much fluctuation in the RMSF graphs obtained shows that the ligands bind tightly to the receptor and form a stable structure (Akash et al., 2024). When the RMSF graphs are examined, it is generally observed that these natural ligands form a stable structure with the APC receptor, which is associated with colorectal cancer. Therefore, they show promise as therapeutic natural agents in the treatment of colorectal cancer (Ye et al., 2023). However, it should be taken into consideration that the RMSF values increase in atoms around 800 to 1000 and 4600 in all ligand complexes and that the structures

undergo conformational changes in certain regions (Wankowicz, de Oliveira, Hogan, van den Bedem, & Fraser, 2022).

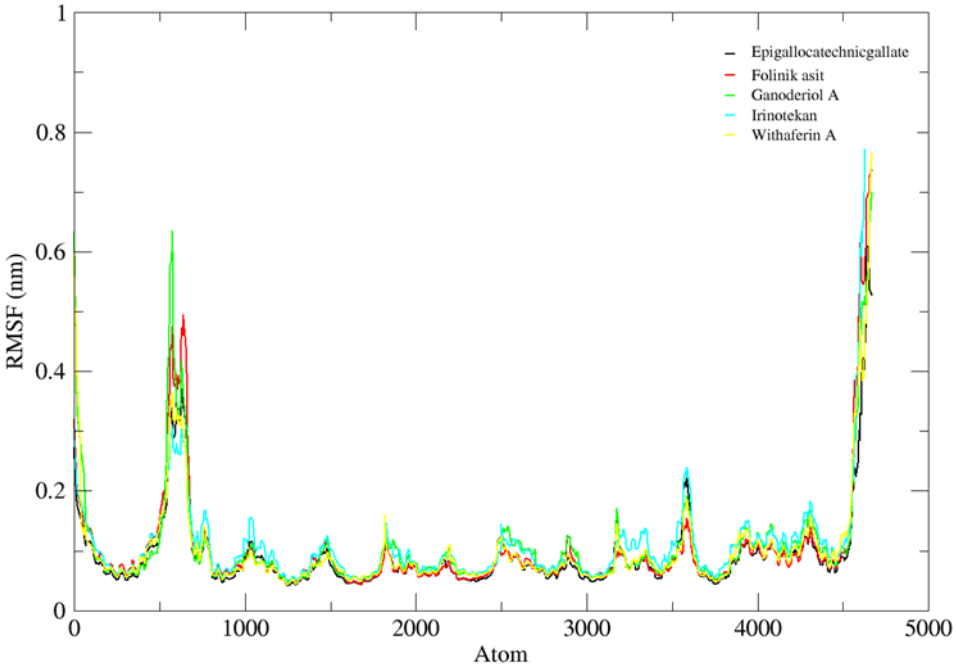

Figure 9. RMSF graph between the backbone of the receptor and ligands of epigallocatechin gallate, folinik asit, ganoderiol A, irinotecan, and withaferin A, respectively.

The term Radial Distribution Function (RDF) represents the average density of a reference molecule or atom of other molecules and atoms at a certain distance (Saha et al., 2024). This type of function is used in molecular dynamics simulations to analyze the distribution and concentration behavior of the ligand at certain distances in receptor-ligand complexes (Shariatinia & Mazloom-Jalali, 2020). When the RDF graphs of the complexes obtained with the APC receptor and the ligands EGCG, folinic acid, ganoderiol A, irinotecan, and withaferin A in Figure 10 are examined, sharp peaks are generally not seen. However, the peak values are generally at 1 nm and higher. This shows that the ligands bind to the receptor compactly at these peak values (Saha et al., 2024). When the RDF values of the EGCG complex are examined, it is seen that this ligand has a tight binding feature with the protein and a high density around 1.5 nm. Although folinic acid does not have a sharp peak, its RDF values, where it binds strongly to the receptor, are between 1 and 2 nm. The ganoderiol A ligand complex gives clearer results compared to other ligands. The peak is around 1.2 nm and shows the highest RDF value. Its high RDF value indicates that it binds to the APC receptor in a very strong and stable structure (Shariatinia & Mazloom-Jalali, 2020). The RDF values of irinotecan and withaferin A ligand complexes are much lower than other ligand complexes. This indicates that these ligands bind less stable and weakly to the receptor (Saha et al., 2024). However, these values are generally average. The reason why ligands do not have sharp peaks in RDF graphs may be due to

their structures. In addition, factors such as ligands interacting on a wide surface in different regions of the receptors, flexible structures, or multiple binding modes may also cause this situation (Saha et al., 2024; Shariatinia & Mazloom-Jalali, 2020). As a result, ganoderiol A ligand gave the best RDF value and graph for the APC receptor.

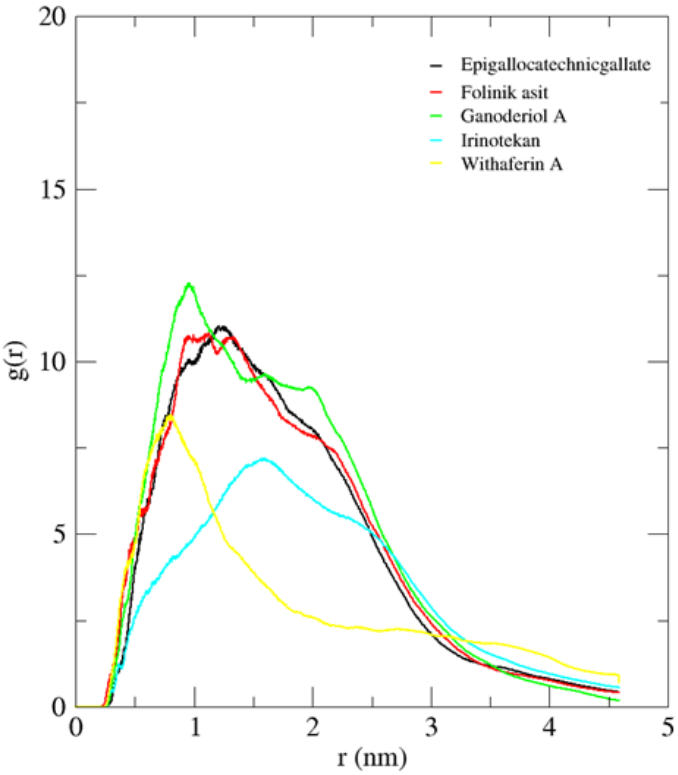

Figure 10. RDF graph between the backbone of the receptor and ligands of epigallocatechin gallate, folinik asit, ganoderiol A, irinotekan and, withaferin A, respectively.

Rg measures the average distance of the atoms to be calculated from the center of mass of a molecule structure (Rampogu, Lee, Park, Lee, & Kim, 2022). Within the scope of molecular dynamics simulation, Rg plots are used to analyze the stability and compactness of the protein-ligand complex structure (Hephzibah Cathryn & George Priya Doss, 2023). While low Rg values indicate that the complex structure is stable and compact, high Rg values indicate that the binding affinities of the protein and ligand are not stable (Justino, Nascimento, & Justino, 2021).

When the RG graphs of the APC receptor and five different ligand complexes in Figure 11 are examined, it was stated that all ligand complex structures generally exhibit low and stable values. The RG value of each ligand complex with the APC gene remains approximately 2.3 nm throughout the simulation. This indicates that each ligand, whether natural or chemical, forms a compact complex with the APC gene. A minimal increase in RG value after 15000 ps suggests that the ligand structure is attempting to find optimal stability on the receptor. Data obtained from graphs such as RG are crucial for determining the structural stability of natural agents with potential as therapeutic drugs in

the treatment of serious diseases, such as colorectal cancer. Identifying the most stable natural ligand complexes with the lowest RG values highlights the potential of these natural ligands as drug candidates (Rampogu et al., 2022). In general, while each ligand-receptor complex structure shows high compactness in the RG graphs, naturally derived EGCG was found to be as robust as the chemical drugs used in colorectal cancer treatment, along with complexes formed with the natural ligands Ganoderiol A and Withaferin A. These ligands can be selected as natural agents with therapeutic potential for the treatment of colorectal cancer and can guide research on new drugs derived from natural sources.

When the results of this literature study were compared with previous studies, it was observed that ligands such as Withaferin A, EGCG, and Irinotecan have been subjected to molecular docking studies on different receptors. In a study utilizing the ligand Withaferin A, its anti-cancer, anti-diabetic, and cholesterol-lowering effects were investigated. Specifically, Mortalin (HSPA9) with a PDB ID of 4KBO and Nrf2 (NFE2L2) with a PDB ID of 2FLU were examined in the context of cancer. In this study, binding energies of -8.85 kcal/mol and -12.59 kcal/mol were found for the mortalin and Nrf2 receptors, respectively. The results for other diabetes-related receptors ranged from -7 to -5 kcal/mol (Surya Ulhas & Malaviya, 2023). Although there are studies in the literature on ligands such as Ganoderiol D and Ganodermanotriol, the cancer receptor related to the Ganoderiol A ligand has not been studied (Bharadwaj et al., 2020). In a different study, the effects of the EGCG ligand on liver cancer were investigated. To measure the binding affinity of the EGCG ligand, receptors including TNF (Tumor Necrosis Factor), ESR1 (Estrogen Receptor 1), BCL2 (B Cell Lymphoma 2), STAT3 (Signal Transducer and Activator), HIF1A (Hypoxia-Inducible Factor 1A), MMP9 (Matrix Metalloproteinase 9), HSP90AA1 (Heat Shock Protein 90kDa), MMP2 (Matrix Metalloproteinase 2), BRAF (B-Raf Proto-Oncogene), and PIK3CA (Phosphoinositide 3-Kinase Catalytic Subunit) were examined. In this study, binding energies were reported to be lower than -7.0 kcal/mol. This indicates that the EGCG ligand shows a much stronger affinity for the APC gene receptor compared to the results reported in the literature (Yang et al., 2024). Mahalekshmi et al. investigated the effect of the antineoplastic agent irinotecan on target proteins of colorectal cancer using molecular binding analyses. In this study, the Epidermal Growth Factor Receptor (EGFR), Matrix Metalloproteinase (MMP), Serine/Threonine Protein Kinase B (AKT1), BRAF, Tankyrase 1 (TNKS-1), and Tankyrase 2 (TNKS-2) receptors, with PDB IDs 1M17, 2DDY, 3O96, 4MNE, 5ECE, and 6KRO, respectively, were used as receptors. The molecular docking results were -8.70 kcal/mol for the AKT1 gene, -7.62 kcal/mol for TNKS-2, -6.52 kcal/mol for MMP, -5.68 kcal/mol for EGFR, -4.34 kcal/mol for TNKS-1, and -3.54 kcal/mol for BRAF. The results of this literature study indicate that the APC gene and irinotecan form a protein-ligand complex with a strong interaction, with a binding energy of -8.5 kcal/mol (Vallikondaperumal et al., 2023). Overall, in this study conducted

with the APC gene, natural and chemical-based ligands showed similar or sometimes even better binding energies than those reported in the literature. However, since the gene receptors and crystal structures used in literature studies have different structural properties, such as distinct active site structures, surface properties, and amino acid sequences, it is expected that the binding energies obtained from molecular docking with the ligands will differ (C. J. Morris & Corte, 2021).

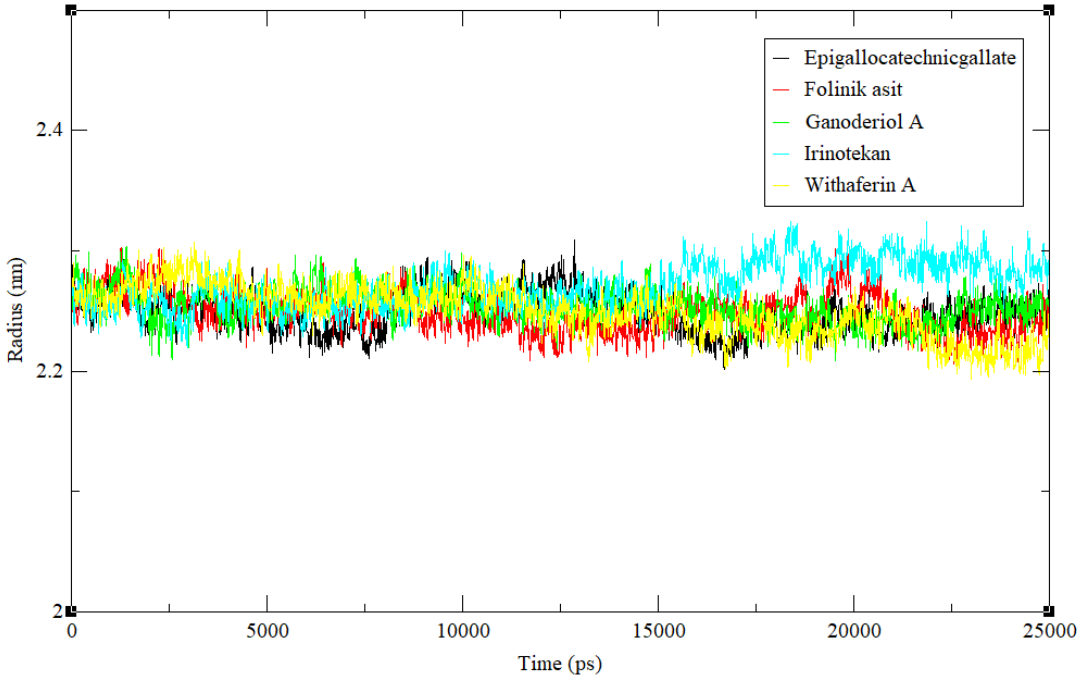

Figure 11. Rg graph between the backbone of the receptor and ligands of epigallocatechin gallate, folinik asit, ganoderiol A, irinotekan and, withaferin A, respectively.

As a result of all the analyses and data obtained within the scope of this study, it was concluded that natural agents are very good potential drug candidates for the treatment of colorectal cancer. These natural agents have fewer side effects, unlike chemical drugs, and will be a ray of hope for many literature studies, especially on colorectal cancer. The main purpose of this study is to investigate compounds with potential therapeutic properties predicted for the APC protein using various molecular modeling approaches, such as molecular docking and dynamic simulation, within an in silico environment. Computational approaches, such as molecular modeling, are employed to assess the drug potential of compounds prior to experimental validation. These methods offer significant advantages in terms of time and cost efficiency before advancing to experimental stages. Furthermore, computational studies conducted in an in silico environment support sustainability and eco-friendly practices by minimizing the consumption of materials and waste typically associated with laboratory experiments. Therefore, analyzing the interactions and pharmacological properties of

the APC protein and its ligands in an in silico environment is crucial for enhancing the efficiency of experimental studies and establishing a robust foundation for subsequent biological validation.

4. Conclusions and Recommendations

This study comprehensively evaluated the potential of natural compounds as alternatives to conventional drugs for colorectal cancer treatment, specifically targeting the APC protein. The analysis included both traditional chemotherapeutic agents, irinotecan and folinic acid, and natural compounds such as withaferin A, EGCG, and ganoderiol A. Using molecular docking studies, the binding affinities of these compounds to the APC protein were assessed, revealing that both natural and conventional drugs exhibited strong interactions.

Irinotecan, which has the best binding affinity score (-8.5 kcal/mol), has proven its anticancer effectiveness in the treatment of colorectal cancer by forming stable hydrogen bonds with the APC protein selected as the receptor. Folinic acid also showed significant binding, supporting its role as a chemotherapeutic adjunct. Among the natural compounds, withaferin A displayed binding affinity comparable to irinotecan, highlighting its potential as a potent therapeutic agent. EGCG and ganoderiol A also formed stable interactions, indicating their promise in cancer treatment.

The SwissADME tool provided valuable predictions of pharmacokinetic properties and drug-likeness. The natural compounds exhibited favorable pharmacokinetic profiles and adhered to Lipinski's rule of five, indicating good oral bioavailability. Withaferin A and ganoderiol A satisfied additional drug-likeness filters (Ghose, Veber, and Egan), reinforcing their suitability as drug candidates. Molecular dynamics simulations further confirmed the stability of the protein-ligand complexes, particularly for withaferin A and ganoderiol A.

The integration of these computational methods offers a robust framework for identifying promising natural compounds for cancer therapy. The results underscore the potential of withaferin A and ganoderiol A as effective alternatives to conventional chemotherapeutic agents, potentially offering treatments with fewer side effects. These findings highlight the importance of continued exploration of natural compounds in oncology, paving the way for innovative and effective therapeutic strategies.

Future research should focus on in vivo studies and clinical trials to validate these computational findings. Investigating the efficacy, safety, and mechanistic pathways of these natural compounds in biological systems will be crucial. Moreover, exploring the combinatorial effects of these natural agents with existing chemotherapies could provide insights into synergistic treatment approaches. The promising results from this study lay a strong foundation for further investigation

and development of natural compounds, potentially revolutionizing colorectal cancer treatment protocols and improving patient outcomes.

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Authors' Contributions

Deniz Karataş: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Investigation, Formal analysis, Resources, Data curation. **Nil Sazlı & Eda Arabacı:** Writing – review & editing, Validation, Investigation, Methodology.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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