Molecular docking interaction of naringin against apoptotic proteins and lung cancer associated proteins

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ABSTRACT

Flavonoids have multiple mechanisms of action and are known to be anticancer agents. Furthermore, flavonoids have been shown to inhibit cancer cell initiation, promotion, and progression by regulating various receptors or enzymes and interfering with signal transduction pathways involved in cell proliferation, differentiation, inflammation, angiogenesis, metastasis, apoptosis induction, and multidrug resistance reversal. Flavonoids and their derivatives have the ability to influence the host immune system, which can be useful incancer treatment.The current work attempted to dock naringin, possible anticancer self -therapeutic flavonoid with apoptotic proteins, Bcl-2, Bax, Caspase-3,Caspase-9 and using the Argusdock tool and determine whether naringin might be employed as cancer medicines. Naringin was also docked with EGFR, ALK2, KRAS, ROS1, lung surfactant proteins (SF-A, SF-D), and ACE2 receptor protein to check whether naringin can target lung cancer. The protein structures were obtained from PDB database, the flavonoid structure was drawn using Avogadro software. The interaction between Naringin and various proteins was examined and it was found that the binding energy values were relatively high. In fact, the binding energy value for Caspase-9 was the highest at -11.0565 kcal/mol, suggesting that Naringin has a strong potential to interact with these proteins and potentially impact their activity. The analysis revealed that Naringin had the highest affinity for ROS1 as indicated by the most negative binding energy value (-10.4575 kcal/mol) among all the proteins. On the other hand, the binding energy of Naringin with ALK was found to be the least negative (-8.62789 kcal/mol) among all the proteins, indicating that Naringin has the lowest affinity for ALK.

Keywords: Naringin, molecular docking, Argus lab, Bcl-2, Bax, Caspase-3, caspase-9, EGFR, ALK2, KRAS, ROS1, lung surfactant proteins, ACE2 receptor protein

1. INTRODUCTION

Lung cancer is the most commonly diagnosed cancer in the world, accounting for 11.4% of all cancer cases in 2020 [1]. It is also the leading cause of cancer death globally, responsible for 18% of all cancer deaths in 2020 [2].The incidence and mortality of lung cancer varies widely across the world, with the highest rates seen in developed countries like the United States and Europe, and lower rates seen in less developed regions like Africa and South Asia [3] Smoking is the primary cause of lung cancer, and the majority of cases are preventable by avoiding tobacco use or exposure to second hand smoke [4] Other risk factors for lung cancer include exposure to air pollution, radon gas, occupational exposure to carcinogens like asbestos and diesel exhaust, and a family history of lung cancer [5]. "While lung cancer has a relatively high incidence compared to other cancers, it has a lower 5-year survival rate (around 20%) compared to many other cancers [6]. For example, the 5-year survival rate for breast cancer is over 90% [7], while the 5-year survival rate for prostate cancer is around 98% [8].Unlike some other types of cancer, there are no routine screening tests for lung cancer that are recommended for the general population [9].

Flavonoids are a diverse group of naturally occurring polyphenolic compounds that are found in various plant-based foods, such as fruits, vegetables, tea, and wine [10].They are known for their antioxidant and anti-inflammatory properties, as well as their potential health benefits, including reducing the risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders[11]

Flavonoids are widely distributed in the plant kingdom and are classified into six main subclasses: flavonols, flavones, flavanones, flavan-3-ols (also called catechins), anthocyanins, and isoflavones. These subclasses differ in their chemical structure and the type and number of hydroxyl and other functional groups attached to their molecular framework [12]

Flavonoids have been extensively studied for their health-promoting effects, and a large body of research has shown that they possess a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [11]. These properties are believed to be due to their ability to scavenge free radicals, inhibit pro-inflammatory enzymes, and modulate various signaling pathways in the body [13].

Many epidemiological studies have investigated the association between flavonoid intake and the risk of chronic diseases, and the results suggest that higher intake of flavonoids is associated with a lower risk of cardiovascular disease, cancer, and neurodegenerative disorders. However, the exact mechanisms by which flavonoids exert their health benefits are still not fully understood[14]

Flavonoids have been shown to have potential anticancer effects, including against lung cancer. Lung cancer is a major cause of cancer-related deaths worldwide, and there is a need for new approaches to prevent and treat this disease.

Previous research has suggested that certain flavonoids, such as quercetin, kaempferol, and apigenin, may have chemopreventive effects against lung cancer. For example, in a study of

over 2,500 individuals in the Netherlands, higher intake of flavonoids was associated with a lower risk of lung cancer [15]. Similarly, a study of over 1,300 lung cancer patients in China found that higher dietary intake of flavonoids was associated with a lower risk of lung cancer recurrence and improved overall survival [16].

In vitro and animal studies have also provided evidence for the potential anticancer effects of flavonoids against lung cancer. For example, in a study using a mouse model of lung cancer, treatment with the flavonoid luteolin inhibited tumor growth and metastasis [17]. Similarly, in vitro studies have shown that quercetin and kaempferol can induce cell cycle arrest and apoptosis in lung cancer cells [18, 19].

Naringin is a flavonoid glycoside commonly found in citrus fruits, such as grapefruit and oranges. It has been shown to possess a range of potential health benefits, including anticancer properties.

Several studies have investigated the potential anticancer effects of naringin against various types of cancer, including lung cancer. In vitro studies have shown that naringin can inhibit the proliferation and induce apoptosis of lung cancer cells, possibly through mechanisms involving the regulation of key signaling pathways [20, 21].

In addition, animal studies have provided evidence for the potential chemopreventive effects of naringin against lung cancer. For example, in a study using a mouse model of lung cancer, treatment with naringin was found to significantly inhibit tumor growth and improve survival [22].

Apoptosis is a natural process by which cells in the body die in a controlled manner, and it is important for maintaining tissue homeostasis and eliminating cells that are no longer needed. Bcl-2 and Bax are two important proteins that regulate apoptosis along with caspases. Bcl-2 is an anti-apoptotic protein that is located on the outer membrane of mitochondria, which are the energy-producing organelles within cells. Bcl-2 inhibits apoptosis by preventing the release of cytochrome c from the mitochondria, which is a key step in the apoptotic pathway. In contrast, Bax is a pro-apoptotic protein that is also located on the outer membrane of mitochondria. Bax promotes apoptosis by promoting the release of cytochrome c from the mitochondria, which activates caspases and ultimately leads to cell death.The balance between Bcl-2 and Bax is critical for determining whether a cell will undergo apoptosis or survive. Caspase-3 and caspase-9 are enzymes involved in programmed cell death, also known as apoptosis. Caspase-3 is responsible for the execution of apoptosis, while caspase-9 initiates the process by activating caspase-3.ALK2 (Activin receptor-like kinase 2) is a type I receptor serine/threonine kinase belonging to the TGF-β receptor superfamily. It plays a role in several signaling pathways, including bone morphogenetic protein (BMP) signaling. KRAS is a gene that encodes a protein called KRAS, which is a member of the Ras family of small GTPases. KRAS is involved in the regulation of cell division and growth, and mutations in this gene are commonly found in various cancers. EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase that plays a critical role in the regulation of cell growth and proliferation. Mutations in the EGFR gene have been identified in various types of cancer, including lung cancer. ROS1 (c-ros oncogene 1) is a receptor tyrosine kinase that plays a role in the regulation of cell growth and differentiation. Mutations in the ROS1 gene have been identified in several types of cancer, including non-small cell lung cancer.

Lung surfactant proteins are essential components of the lung surfactant system, which helps to reduce surface tension in the lungs and prevent alveolar collapse during breathing. There are four main types of lung surfactant proteins: surfactant protein A (SP-A), surfactant protein B (SP-B), surfactant protein C (SP-C), and surfactant protein D (SP-D).

SP-A and SP-D are members of the collectin family, which are proteins that bind to and help clear pathogens from the lungs. These proteins also have important roles in modulating the immune response in the lung and regulating inflammation [23].

SP-B and SP-C are hydrophobic proteins that play key roles in stabilizing the lung surfactant film at the air-liquid interface. SP-B is essential for proper folding and function of another surfactant protein, called surfactant protein C [24].

Mutations in genes encoding lung surfactant proteins can lead to a range of respiratory disorders, including acute respiratory distress syndrome (ARDS), interstitial lung disease, and pulmonary fibrosis [25]. In addition, alterations in the expression or function of these proteins have been implicated in the pathogenesis of several other lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), and lung cancer [26].

There is growing evidence to suggest that lung surfactant proteins may play a role in the development and progression of lung cancer. For example, studies have shown that altered expression of surfactant proteins, particularly SP-A and SP-D, is associated with a higher risk of lung cancer [27,28].

SP-A and SP-D have been found to have both pro- and anti-tumor effects in lung cancer. On one hand, these proteins can promote tumor cell proliferation and invasion by interacting with specific receptors on cancer cells. On the other hand, they can also stimulate immune cells to attack and eliminate cancer cells [29,30].

In addition, mutations in the gene encoding SP-C have been linked to an increased risk of developing lung cancer, possibly through mechanisms involving altered surfactant function and immune regulation [31].

There is some evidence to suggest that lung surfactant proteins SP-A and SP-D may have anti-apoptotic properties in lung cancer. For example, studies have shown that these proteins can interact with certain signaling pathways involved in cell survival and apoptosis, such as the PI3K/AKT and ERK1/2 pathways, and may promote cell survival by inhibiting apoptosis [32,33].

In addition, SP-D has been shown to protect lung cancer cells from chemotherapy-induced apoptosis by upregulating the expression of anti-apoptotic proteins and downregulating the expression of pro-apoptotic proteins [34].ACE2 (Angiotensin-Converting Enzyme 2) is a type I integral membrane protein expressed on the surface of cells in a variety of tissues, including the lungs, heart, kidneys, and gastrointestinal tract. It is a key component of the renin-angiotensin-aldosterone system (RAAS), which plays a critical role in regulating blood pressure and fluid balance in the body [35].

One of the primary functions of ACE2 is to cleave and inactivate angiotensin II, a peptide hormone that constricts blood vessels and raises blood pressure. By cleaving angiotensin II, ACE2 helps to counteract its effects and maintain cardiovascular homeostasis [36].Overall, while more research is needed to fully understand the mechanisms by which flavonoids may exert their anticancer effects against lung cancer, the existing evidence suggests that these compounds may have promise as a preventive and therapeutic approach to this disease.

2. Methodology

In this study, in-silico research was conducted using the Argus lab tool. To prepare the protein and ligand, we utilized the Avogadro software. Additionally, the receptor-ligand docking process was performed with the Argus lab tool. Furthermore, we used the Swiss ADME server to predict the drug likeness of all compounds that were selected for this study.

2.1 Selection of target protein and Preparation of protein target structure

The target protein receptors for this study were obtained from the RCSB Protein Data Bank database and were used for the docking process. To prepare the input files, we utilized the Argus lab software. To ensure accuracy, all molecules of water, miscellaneous residues, and ions were completely removed from the protein receptors. Furthermore, hydrogen atoms were added to the receptors to optimize the docking process.

2.2. Selection and preparation of Ligand molecule

The selected ligand Naringin 3d structure was determined using Avogadro an advanced semantic chemical editor, visualization, and analysis platform. The 3d structure was uploaded into the Argus lab software and the 'correction' option has been used to rectify the torsion tree, non-polar hydrogens, charges, and atom type. With the structure further ADME predictions were carried out by using SWISS ADME to check for drug likeness and toxicity.

2.3. Molecular docking investigation

Molecular docking analysis performed with the potential targets like Bcl_2, bax, caspase3,caspase9, SF-A, SF-D, ACE2. Argus lab software was used for autodock purposes. Here first we uploaded the targeted protein structure as macromolecule, then removed water molecules and miscellaneous residues. After after selecting all the amino acids they were assigned as a binding site group and then selected bioactive compound Naringin (ligand) was uploaded and water molecules were removed and here the residues were assigned as ligand site. Software first minimized ligand energy and then converted it into Autodock ligand format (pdbqt). Finally started blind docking after covering the entire protein structure under grid box to screen best fitted bioactive compounds based on energy value. Each simulation was conducted 5 times, resulting in 5 docked conformations. The least energy configurations were deemed to be the highest binding conformations as a result of this. To achieve better ligand binding settings for 100 individual LGA variants with a greatest number of critical forecasts of 25000000, this docking methodology used an overall population of 300 and a largest frequency of assessments of 27,000. Using Argus lab, the intra - molecular interactions such as hydrogen bonds, van der Waals, and hydrophobic interactions with specific bioactive compound have been clearly analysed.

3. Results

Molecular docking is a computational method used to predict how small molecules, such as drugs or natural compounds, interact with proteins or other macromolecules in the body. It can provide insights into the potential binding modes and affinity of small molecules with specific proteins, which can be useful for drug discovery and development.

Bcl-2 and Bax are important apoptotic regulatory proteins, with Bcl-2 promoting cell survival and Bax promoting cell death. Caspase-3 and caspase-9 are enzymes involved in the execution phase of apoptosis, playing important roles in the programmed cell death pathway.

Based on the molecular docking analysis using Argus lab software, it was found that Naringin exhibited high affinity towards the apoptotic proteins Bcl-2, Bax, Caspase-3, and

Caspase-9. The results are summarized in Table 1 (figure1), which shows the PDB IDs, number of bonds, bond lengths, interacting residues, and binding energy values for each protein.

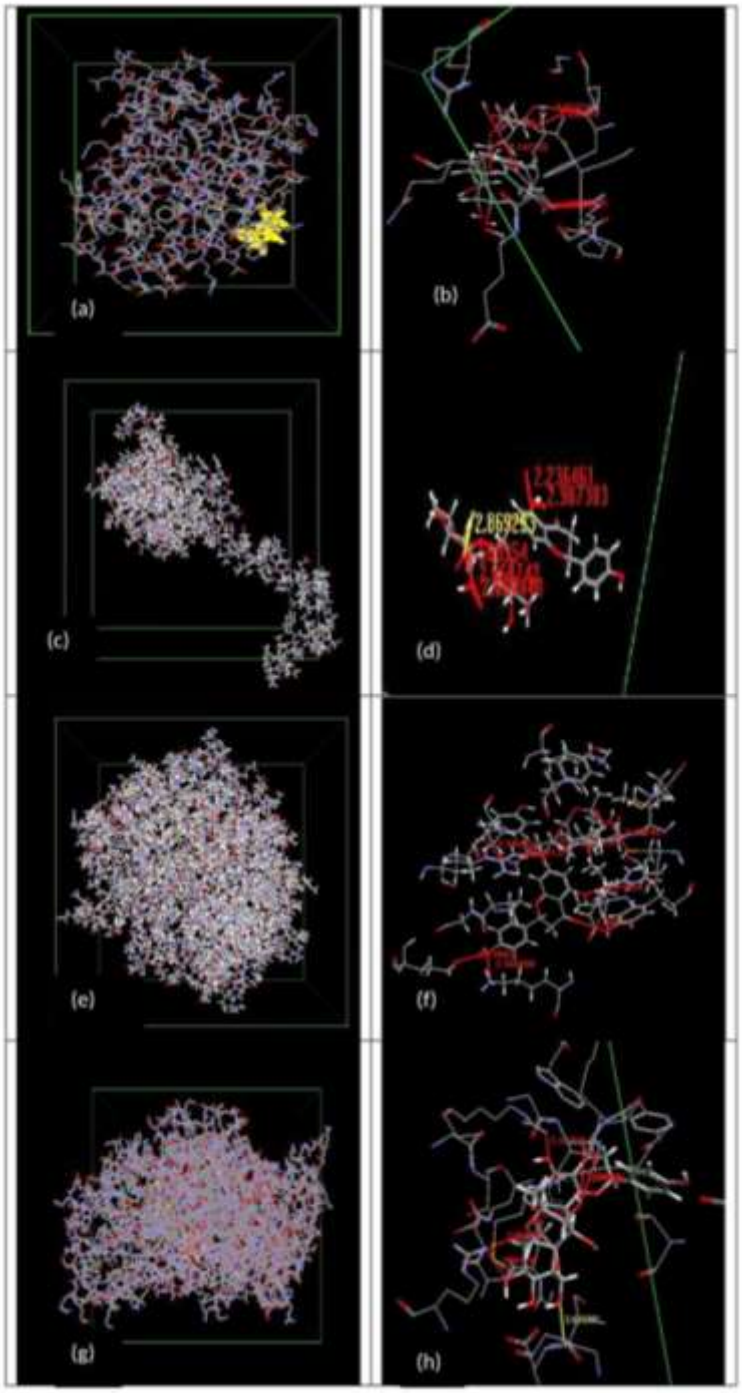
In particular, the binding energy values for the interactions between Naringin and these proteins were relatively high, with Caspase-9 showing the highest value at -11.0565 kcal/mol. This indicates a strong potential for Naringin to interact with these proteins and potentially affect their activity. The binding energy of naringin with ROS1 is the most negative (-10.4575 kcal/mol) among all the proteins listed in the table, indicating that naringin has the highest affinity for ROS1. On the other hand, the binding energy of naringin with ALK is the least negative (-8.62789 kcal/mol) among all the proteins, indicating that naringin has the lowest affinity for ALK (Figure 2).

Table1: Naringin interaction study with pro-apoptotic and antiapoptotic proteins. Interaction energy, interaction residues as well as number of hydrogen bonds involved in interaction are also depicted in the table

Naringin interaction with Proteins	RCSB PDB IDs	NO. of Bonds	Bond length (A∘)	Interacting residues		Binding energy (kcal/mol)
				Atom 1	Atom2	
Bcl-2	6FBX	6	H1: 2.874741 H2: 2.899878 H3: 2.323904 H4: 2.747126 H5: 2.270975 H6: 2.960848	1361(O) in residue173UNK 1360 (O) in residue 173UNK 219(N) in residue 28GLN 219 (N) in residue 28GLN 307 (N) in residue 40ARG 307(N) in residue 40ARG	179(O) in residue 23PHE 179 (O) in residue 23PHE 13650 (O) in residue 173UNK 1360 (O) in residue173UNK 1368 (O) in residue 173UNK 1367(O) in residue173UNK	-8.23323
Bax	4ZIE	4	H1: 2.907383 H2: 2.236461 H3:2.860742 H4:2.869293	2657(O) in residue 190UNK 1372 (N) in residue 111 Val 2653 (O) in residue 190UNK 185(N) in residue 24 ALA	1304(O) in residue 107TRP 2657(O) in residue 190UNK 109(O) in residue 19 ILE 2653 (O) in residue 190 UNK	-9.41795
Caspase3	5JFT	8	H1: 2.586967 H2: 2.961227 H3: 2.898981	1414(N) in residue 128 GLY 2027 (N) in residue 167 ARG 7442 (O) in	74470 (O) in residue 524 UNK 7441 (O) in residue 524 UNK 5069 (O) in residue	-9.91572

			H4: 2.782491 H5: 2.574659 H6: 2.888512 H7: 2.739027 H8: 2.560000	residue 524 UNK 7446 (O) in residue 524 UNK 5690 (N) in residue 415 ARG 6049 (O) in residue 438 TYR 7451 (O) in residue 524 UNK 5253 (O) in residue 388 LYS	375 GLU 7096 (S) in residue 505 CYS 7449 (O) in residue 524 UNK 7450 (O) in residue 524 UNK 2070 (O) in residue 170 GLU 7451 (O) in residue 524UNK	
Caspase9	2AR9	7	H1: 2.277028 H2: 2.774931 H3: 2.900435 H4: 2.592277 H5: 2.564068 H6: 2.997138 H7: 2.662281	3338 (N) in residue 616ARG 7153 (O) in residue1060 UNK 7153 (O) in residue1060UNK 63(N) in residue 148ASN 5286 (N) in residue 868 ARG 5286 (N) in residue 868 ARG 7154 (O) in residue1060 UNK	7156 (O) in residue 1060 UNK 6970(O) in residue 1087 ILE 6990(O) in residue 1089 LYS 7148(O) in residue 1060 UNK 7145(O) in residue 1060 UNK 7149(O) in residue 1060 UNK 4603 (O) in residue 783 GLY	-11.0565

Figure 1: Naringin interaction study with pro-apoptotic and anti-apoptotic proteins.



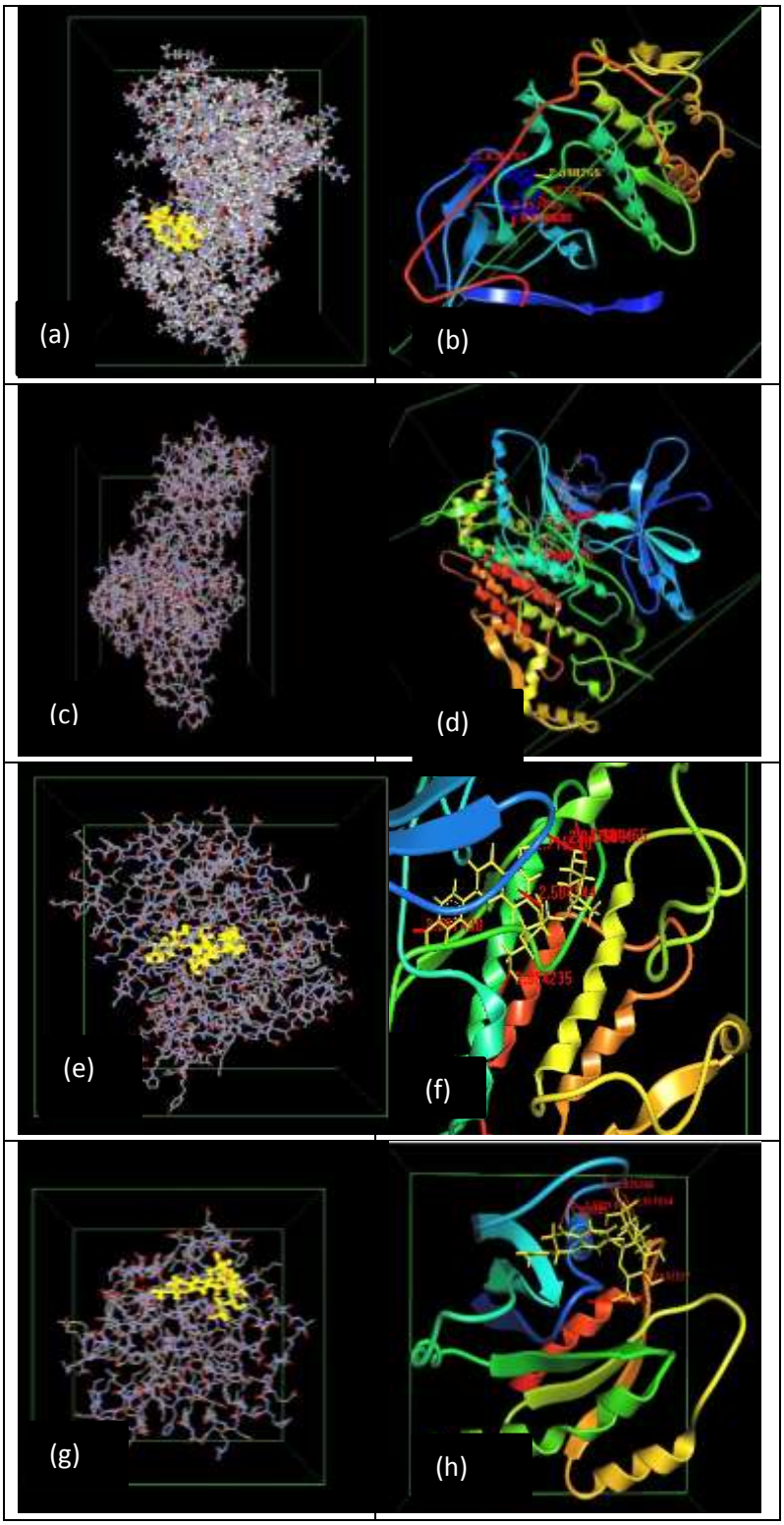
(a) Bcl2-Naringin interaction (b) Bcl2- Naringin bonding (c) Bax-Naringin interaction (d) Bax- Naringin bonding (d) Caspase3-Naringin interaction (e) Caspase3-Naringin bonding (f) Caspase9-Naringin interaction (g) Caspase9-Naringin bonding

Table2: Naringin interaction study with potential lung cancer markers. Interaction energy, interaction residues as well as number of hydrogen bonds involved in interaction are also depicted in the table.

Naringin interaction with Proteins	RCSB PDB IDs	NO. of Bonds	Bond length (A∘)	Interacting residues		Binding energy (kcal/mol)
				Atom 1	Atom2	
EGFR	2GS2	7	H1: 2.455831 H2: 2.257686 H3: 2.552592 H4: 2.197350 H5: 2.898255 H6: 2.835192 H7: 2.942342	4901 (O) in residue 332 UNK 1474 (N) in residue769 MET 1429 (O) in residue 766 THR 2495 (O) in residue 830 THR 4896 (O) in residue 332 UNK 4903(O) in residue 332 UNK 782 (N) in residue 721 LYS	1441(O) in residue 767 GLN 4902 (O) in residue 332 UNK 4901 (O) in residue 332 UNK 4895 (O) in residue 332 UNK 2511 (O) in residue 831 ASP 375(O) in residue 694 LEU 4890(O) in residue 332 UNK	-9.23565
JAK2	4C61	6	H1: 2.966587 H2: 2.347423 H3: 2.976353 H4: 2.927654 H5: 2.675612 H6: 2.211183	4342 (O) in residue 757 UNK 4343 (O) in residue 757 UNK 4343 (O) in residue 757 UNK 3225 (N) in residue 1259 ARG 3225 (N) in residue 1259 ARG 4346(O) in residue 757 UNK	4279 (O) in residue 1390 ASP 4279 (O) in residue 1390 ASP 2966 (O) in residue 1228 SER 4344(O) in residue 757 UNK 4340(O) in residue 757 UNK 235 (O) in residue 877 GLU	-10.0859
ALK	4MKC	6	H1: 2.901465 H2: 2.847509 H3: 2.716528 H4: 2.584344 H5: 2.897430	2394(O) in residue 484UNK 247 (N) in residue 1126 ALA 2397 (O) in residue 484UNK 2390 (O) in residue 484UNK 2399 (O) in residue	250 (O) in residue 1126 ALA 2393 (O) in residue 484UNK 1322 (O) in residue 1270 ASP 236 (O) in residue 1124 HIS 776 (O) in residue	-8.62789

			H6: 2.924235	484UNK 2395 (O) in residue 484UNK	1199 MET 800 (O) in residue 1203 ASP	
KRAS	5P21	6	H1: 2.835268 H2: 2.917534 H3: 2.502135 H4: 2.355885 H5: 2.400354 H6: 2.637227	1331 (O) in residue 382 UNK 1332 (O) in residue 382 UNK 1335 (O) in residue 382 UNK 244 (O) in residue 32 TYR 1330 (O) in residue 382 UNK 982 (N) in residue 117 LYS	227 (O) in residue 31 GLU 212 (O) in residue 29 VAL 244 (O) in residue 32 TYR 1336 (O) in residue 382 UNK 87 (O) in residue 12 GLY 1329 (O) in residue 382 UNK	-9.22995
ROS1	5LAX	7	H1: 2.764463 H2: 2.553806 H3: 2.812712 H4: 2.919228 H5: 2.649828 H6: 2.999640 H7: 2.849169	6310 (O) in residue 1039 UNK 6309 (O) in residue 1039 UNK 6309 (O) in residue 1039 UNK 6312 (O) in residue 1039 UNK 6307 (O) in residue 1039 UNK 4786 (N) in residue 568 ARG 6314 (O) in residue 1039 UNK	4848 (O) in residue 1039 UNK 4848 (O) in residue 574 GLU 4835 (O) in residue 573 GLN 668 (O) in residue 83 THR 4857 (O) in residue 574 GLU 6307 (O) in residue 1039 UNK 4201(O) in residue 514 GLU	-10.4575
Lung SF-A	5FFS	7	H1: 2.787738 H2: 2.785401	1114 (O) in residue 286UNK 266 (O) in residue 120SER	198 (O) in residue110SER 1108 (O) in residue 286UNK	-9.41967

			H3: 2.530384 H4: 2.564733 H5: 2.465798 H6: 2.418270 H7: 2.740659	1109 (O) in residue 286UNK 1112 (O) in residue 286UNK 1112(O) in residue 286UNK 1113 (O) in residue 286UNK 1116 (O) in residue 286UNK	270 (O) in residue 121THR 264 (O) in residue 120SER 1083 (S) in residue 226CYS 1083 (S) in residue 226CYS 403 (O) in residue 138ALA	
Lung SF-D	1B08	4	H1: 2.902505 H2: 2.425596 H3: 2.906222 H4: 2.891344	3456 (O) in residue 655UNK 3455 (O) in residue 655UNK 2835 (N) in residue 2276 GLU 2887 (N) in residue 2282GLN	2577 (O) in residue 240VAL 2824 (O) in residue 273SER 3455 (O) in residue 655UNK 3464 (O) in residue 655UNK	-7.96789
ACE2	1R42	4	H1:2.900362 H2:2.999496 H3:2.691927 H4:2.854963	9531 (O) in residue 616 UNK 6650 (N) in residue 441 LYS 9522(O) in residue 616 UNK 9524(O) in residue 616 UNK	6534 (O) in residue453 GLU 9527 (O) in residue 616 UNK 6104 (O) in residue 406 GLU 5586 (O) in residue371THR	-10.7283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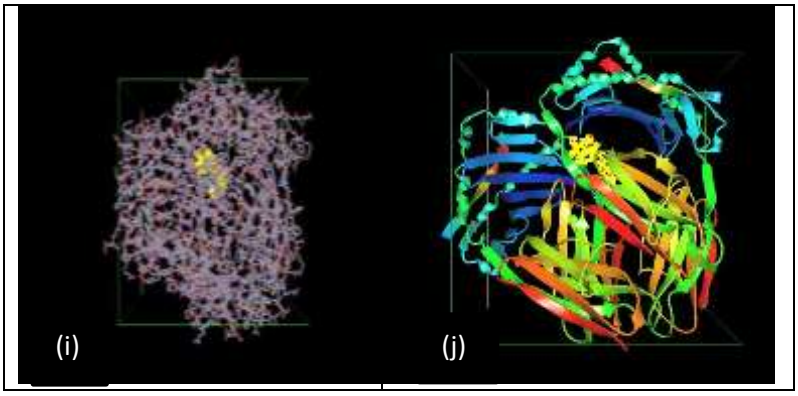
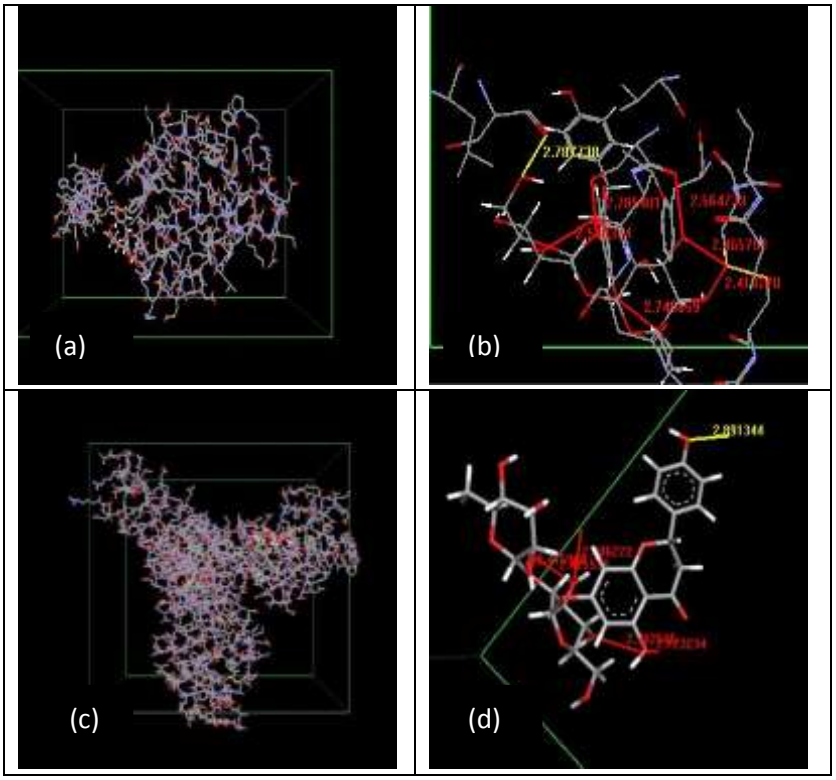
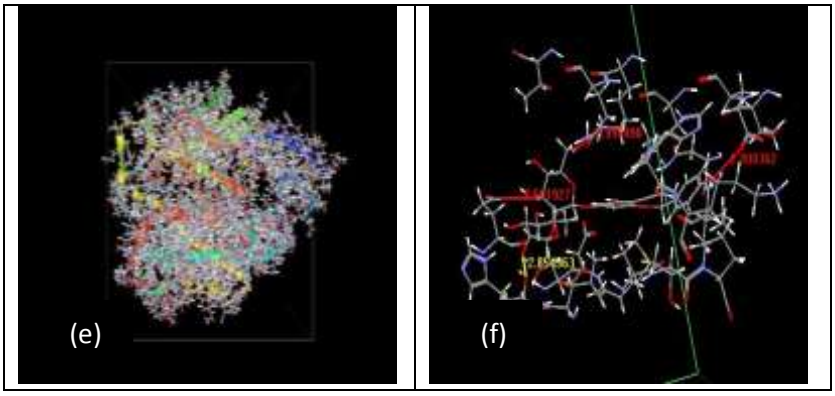


Figure 2: Naringin interaction study with potential lung cancer markers.
(a) EGFR-Naringin interaction (b) EGFR-Naringin bonding (c) JAK2-Naringin interaction (d) JAK2-Naringin bonding (e) ALK- Naringin interaction (f) ALK- Naringin bonding (g) KRAS- Naringin interaction (h) KRAS- Naringin bonding

Figure 3: Naringin interaction study with lung surfactant proteins and ACE2 receptor protein.





(a) Lung surfactant A- Naringin interaction (b) Lung surfactant A- Naringin bonding (c) Lung surfactant D- Naringin interaction (d) Lung surfactant D- Naringin bonding (e) ACE2- Naringin interaction (f) ACE2- Naringin bonding

4.Discussion

Naringin, a flavonoid found in citrus fruits, has been shown to have potential anticancer effects by regulating various signaling pathways involved in cell proliferation, apoptosis, and inflammation. Several studies have investigated the potential role of naringin in regulating the expression of Bcl-2, Bax, caspase-3, and caspase-9 in cancer cells.

Bcl-2 is an anti-apoptotic protein that promotes cell survival by inhibiting apoptosis, while Bax is a pro-apoptotic protein that promotes apoptosis. The balance between Bcl-2 and Bax is critical for regulating apoptosis in cancer cells. Naringin has been shown to induce apoptosis in various cancer cells, including lung cancer cells, by regulating the expression of Bcl-2 and Bax. For example, one study showed that naringin could increase Bax expression and decrease Bcl-2 expression, leading to increased apoptosis in lung cancer cells (37).

Caspase-3 and caspase-9 are key enzymes involved in the apoptotic signaling pathway. Activation of caspase-3 and caspase-9 results in the cleavage of various cellular proteins, leading to apoptosis. Naringin has been shown to induce apoptosis in cancer cells by activating caspase-3 and caspase-9. For example, one study showed that naringin could increase the activity of caspase-3 and caspase-9 in lung cancer cells, leading to increased apoptosis (38).These studies suggest that naringin may have potential as a therapeutic agent for the treatment of lung cancer by regulating the expression of Bcl-2, Bax, caspase-3, and caspase-9. EGFR is frequently overexpressed or mutated in lung cancer, and its activation plays a crucial role in the proliferation, survival, and metastasis of cancer cells. Several studies have shown that naringin can inhibit the growth and induce apoptosis of non-small cell lung cancer cells by regulating the miR-21/PTEN/PI3K/Akt and EGFR/MEK/ERK signaling pathways [39](Li et al., 2019; Chen et al., 2019).

In a study by Li et al. (2019), naringin was found to inhibit the proliferation and induce apoptosis of non-small cell lung cancer cells by downregulating the expression of EGFR and its downstream signaling molecules, including phosphorylated MEK, ERK, and Akt. In addition, naringin was found to upregulate the expression of miR-21, which then downregulated the expression of PTEN, a tumor suppressor gene that negatively regulates the PI3K/Akt signaling pathway. This led to decreased cell proliferation and increased apoptosis.

Another study by Chen et al. (2019) showed that naringin can inhibit the growth and induce apoptosis of non-small cell lung cancer cells by regulating the EGFR/MEK/ERK signaling pathway. In this study, naringin was found to downregulate the expression of EGFR and its downstream signaling molecules, including phosphorylated MEK and ERK. This resulted in decreased cell proliferation and increased apoptosis [40].These findings suggest that naringin has potential as a therapeutic agent for lung cancer by targeting the EGFR signaling pathway.

The JAK2 and ALK genes are frequently altered in different types of cancer, including lung cancer. The activation of JAK2 and ALK signaling pathways can promote tumor cell survival, proliferation, and metastasis. Several studies have explored the interaction between naringin and JAK2/ALK signaling pathways in cancer cells.

In a study by Chen et al. (2018), naringin was found to inhibit the growth and induce apoptosis of lung cancer cells by downregulating the expression of JAK2 and its downstream signaling molecules, including phosphorylated STAT3 and AKT. The authors suggested that naringin could suppress the JAK2/STAT3 signaling pathway, thereby inhibiting the proliferation and promoting apoptosis of lung cancer cells [41].

In another study by Yu et al. (2020), naringin was found to inhibit the proliferation and induce apoptosis of ALK-positive lung cancer cells by downregulating the expression of ALK and its downstream signaling molecules, including phosphorylated AKT, ERK, and STAT3. The authors suggested that naringin could suppress the ALK/PI3K/AKT and ALK/ERK/STAT3 signaling pathways, thereby inhibiting the growth and promoting apoptosis of ALK-positive lung cancer cells [42].

These studies suggest that naringin has potential as a therapeutic agent for lung cancer by targeting the JAK2/STAT3 and ALK/PI3K/AKT/ERK/STAT3 signaling pathways.

The KRAS and ROS1 genes are also frequently altered in lung cancer. KRAS mutations are present in about 25% of non-small cell lung cancers (NSCLC) while ROS1 rearrangements are found in about 1-2% of NSCLC cases. These genetic alterations lead to the activation of downstream signaling pathways, promoting cell growth and survival. Several studies have explored the interaction between naringin and KRAS/ROS1 signaling pathways in cancer cells.

In a study by Li et al. (2019), naringin was found to inhibit the proliferation and induce apoptosis of KRAS-mutant NSCLC cells by downregulating the expression of KRAS and its downstream signaling molecules, including phosphorylated MEK and ERK. The authors suggested that naringin could suppress the KRAS/MEK/ERK signaling pathway, thereby inhibiting the proliferation and promoting apoptosis of KRAS-mutant NSCLC cells [43].

In another study by Li et al. (2020), naringin was found to inhibit the proliferation and induce apoptosis of ROS1-rearranged NSCLC cells by downregulating the expression of ROS1 and its downstream signaling molecules, including phosphorylated AKT and ERK. The authors suggested that naringin could suppress the ROS1/PI3K/AKT and ROS1/ERK signaling pathways, thereby inhibiting the growth and promoting apoptosis of ROS1-rearranged NSCLC cells [44].

These studies suggest that naringin has potential as a therapeutic agent for lung cancer by targeting the JAK2/STAT3, ALK/PI3K/AKT/ERK/STAT3 KRAS/MEK/ERK and ROS1/PI3K/AKT/ERK signaling pathways.

Lung surfactant proteins A and D (SP-A and SP-D) play an important role in the innate immune response of the lung. SP-A and SP-D are known to interact with a variety of pathogens, allergens, and other foreign particles, and facilitate their clearance from the lung. Several studies have explored the interaction between lung surfactant proteins (SP-A and SP-D) and ACE2 in the context of lung cancer. In a study by Wang et al. (2019), the authors found that SP-A and SP-D can interact with ACE2 and modulate its expression and activity in lung cancer cells. The authors suggested that SP-A and SP-D could regulate the RAAS pathway and promote the development and progression of lung cancer by modulating ACE2 activity [45].

In another study by Zhang et al. (2020), the authors found that SP-D can directly interact with lung cancer cells and promote their proliferation and migration. The authors suggested that SP-D could enhance the invasion and metastasis of lung cancer cells by interacting with integrin αvβ3 and activating the AKT signaling pathway [46]. These studies suggest that lung surfactant proteins and ACE2 may play a role in the development and progression of lung cancer by modulating the RAAS pathway and promoting cell proliferation, migration, and invasion.However, it should be noted that additional studies are necessary to validate the aforementioned findings and further elucidate the underlying mechanisms of the interaction between naringin and above mentioned proteins in the context of lung cancer.

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