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RESEARCH ARTICLE OPEN ACCESS

"In-silico evaluation, analysis and screening of a potent polyphenolic herbal drug molecule and its validation as a candidate drug inhibiting p100/RELB domain of NF-kB alternate pathway through molecular docking studies."

Aman[1], Nidhi[2]

[1]University Institute of Biotechnology, Chandigarh University, Mohali, Punjab-140413, India.

Email-kaushikaman0405@gmail.com,

[2] University Institute of Biotechnology, Chandigarh University, Mohali, Punjab-140413, India.

Email-nidhichhikara95@gmail.com,

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Abstract

Upregulation of various flagging pathways is required for the uncontrolled tumor cell expansion. Mitogen enacted protein and NF-kB pathways have been watched for demonstrating exceptionally critical impacts by the oxidants. Cell expansion is advanced by the outflow of NF-kB and its restraint squares cell multiplication.A large portion of the herbs utilized as hostile to carcinogenic medications are anticipated to restrain the traditional pathway of the NF-kB for example p65/RELA space. Each one of those herbs have different reactions that might be destructive for future perspectives. Much after restraint of the old style pathway, cell multiplication can go on through the elective pathway. Be that as it may, Silymarin represses the elective pathway of NF-kB by authoritative to p100/RELB space which stifles the kinase compound that phosphorylates the I-kB. The dynamic I-kB hinders the traditional pathway by authoritative to the p65/RELA space and stops its translocation to core further anticipating the DNA translation, which thusly at long last stops cell multiplication.

Keywords: NF-kB, Silymarin, Oxidative stress, p100/RELB domain.

Introduction

In Silico Drug Designing

The expression “In Silico” means performed via computer simulation. Identification of the drug target molecule by employing bioinformatics tools is defined as in siIico drug designing. It helps in identification of drug target, analysis of target structures for active sites, produce candidate molecules, comparing the drug likeness, perform docking with the target, analysing them according to their binding affinities and optimize the molecules for their improved binding characteristics.

Oxidative Stress

Imbalance between production of free radicals/reactive oxygen species ROS and antioxidants is referred to as oxidative stress.Many factors enhance oxidative stress and excess free radical production. These factors can include:

- diet
- lifestyle
- certain conditions
- environmental factors such as pollution and radiation

Oxidative stress can also be triggered by body’s natural immune response. A mild inflammation is caused by this type of oxidative stress that fades away after the immune system fights off an infection or repairs an injury. Body’s cells and major biomolecules gets damaged due to this imbalance and leaves a potential impact on the organism. (Durackova et.al. 2009) Damage of cells, proteins and DNA contribute to aging which is an outcome of oxidative stress. It also plays a role in development of a range of health conditions, including diabetes, cancer, and diseases such as Alzheimer's. Normal cellular metabolism generates ROS through mitochondrial respiratory chain. (Poyton et.al.2009) ROS produced have a major role in simulation of signalling path ways in cells in response to changes in cellular environment conditions.(Jabs et.al. 1999) Hydrogen peroxide, superoxide anion, organic peroxides and hydroxyl radical are the ROS produced by aerobic cells during endogenous metabolic reactions.(Fridovich et.al.1978) Nitric oxides are also produced in the mitochondrial repiratory chain under hypoxic conditions which further generates other reactive species by inducing lipid peroxidation such as 4-

hydroxynonenal and malondialdehyde.(Hussain et.al. 2003) Modification of proteins and lipids can increase the risk of mutagenesis as they are the significant target of oxidative attack.(Schraufstatter et.al. 1988) Oxidative stress is linked to cancer initiation and progression by genome instability, inducing DNA damage, initiation of tumorigenicity and cell proliferation.(Visconti et.al. 2009)

Increased survival ability of the tumor cells as compared to normal cells is its main characteristics. ROS are reported to be tumorigenic as the induction of DNA damage leads to genetic abrasion that initiates tumor and later tumor progression. ROS can also function as anti-tumorigenic agent as it can induce cell death and cell senescence. ROS activity depends on location and concentration ROS production whether to act as anti-tumorigenic agent or to promote tumor cell survival.

Upregulation of multiple signalling pathways is required for the uncontrolled tumor cell proliferation. Mitogen activated protein and NF-kB pathways have been observed for showing highly significant effects by the oxidants.(Muller et.al. 1997) Cell proliferation is promoted by the expression of NF-kB and its inhibition blocks cell proliferation.(Rath et.al. 2001) Tumor necrosis factor is the second messenger implicated by ROS for the activation of NF-kB.(Schulze-Osthoff et.al. 1998)

Nuclear Factor - kappa B (NF-kB)

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that helps in controlling transcription of DNA, production of cytokine and cell survival. It is retained in all animal cell types and is involved in responses to stimuli such as free radicals, stress, cytokines, heavy metals, ultraviolet irradiation, and bacterial or viral antigens.(Sen et.al 1986) NF-κB plays a key role in regulating the immune response to infection. Excessive production of NF-κB has been reported to cause inflammatory and autoimmune diseases, cancer, septic shock, viral infection, and improper immune development.(Mitchell et.al. 2016)

Review of Litrature

Nuclear Factor - kappa B (NF-kB) Pathway

The transcription factor nuclear factor-kb is identified as DNA binding protein in activated B cells(Sen et.al 1986) which modulates gene expression in cellular processes like stress response to various stimuli, cell proliferation, immunity response, apoptosis and organ development.(Hayden et.al. 2004) The NF-kB includes five distinct homo and heterodimer proteins, the NF-kB subfamily proteins – p50, p52 and its precursors p105 and p100 and the REL subfamily proteins – RELA/p65, RELB and c-REL. The classical and alternative pathway are the main signalling pathways that lead to the activation of NF-kB target genes. The classical pathway involves p105 in collaboration with p65 and is triggered by cytokine receptors and the p100 is involved in alternative pathway with RELB and is activated by B cells activating factor. (Mitchell et.al. 2016)

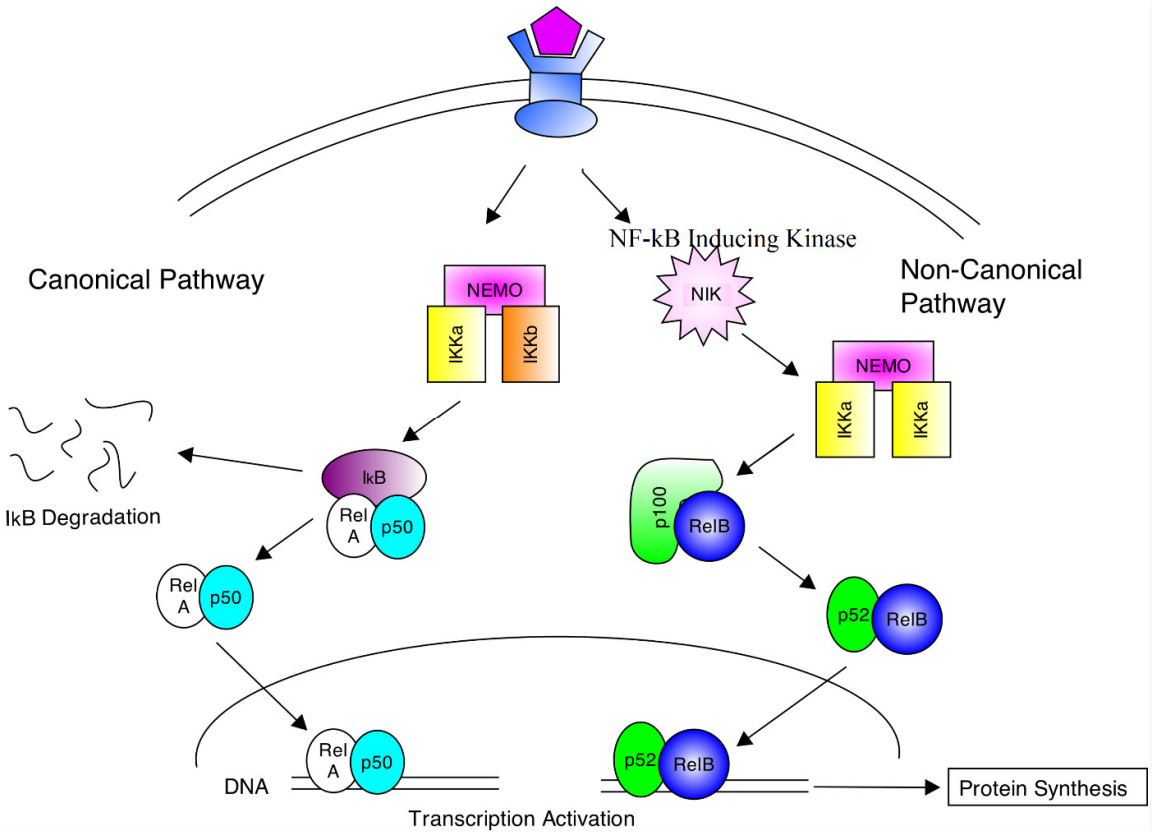


Figure 1: Depicts canonical and non-canonical pathways of NF-kB for the transcripton of DNA for the protein synthesis. (Williams et.al. 2014)

The heterodimer of p50-p65 is the most abundant form and is localized in the cytoplasm. Dimer formation, recognition, DNA binding and interacting with inhibitory kB proteins is the responsibility of REL domain. (Lingappan et.al. 2013) The inhibitory kB proteins REL domain of NF-kB/REL protein, mask its DNA binding site and interfere with their function. The inflammatory signals of oxidative stress induce phosphorylation of inhibitory kB proteins by kinases and leads to degradation of I-kB. Then the target genes for the cell proliferation gets activated by the translocated active NF-kB into the nucleus. (Zhang et.al. 2017)

Relation between Nrf2 and NF-kB pathways:

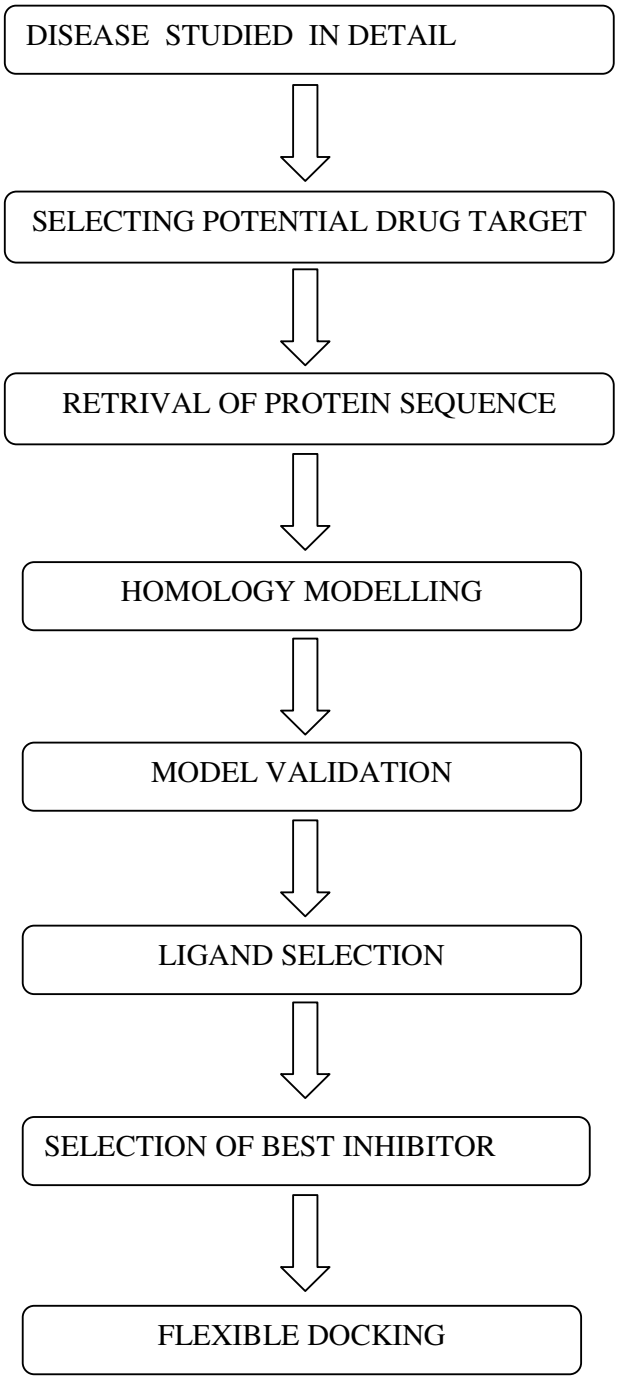
Under oxidative stress conditions, the transcriptional and post-transcriptional mechanism link Nrf2 with NF-kB. Suppression of Nrf2 increases the activity of NF-kB which leads to increased cytokine production. (Pan et.al 2012) In abnormal cell proliferation in free radical chain reaction in humans, abnormal expression of NF-kB is seen which is driven by reduction in Nrf2 activity. (Thimmulappa et.al. 2006) Inducing antioxidants in the body regulates the Nrf2 expression which inhibits the phosphorylation of I-kb proteins and indirectly controls the NF-kB expression.(Liu et.al. 2008)

Effect of oxidative stress on the NF-kB pathway:

The state of oxidative stress is attained when the production of reactive oxidative species is higher than the antioxidant capacity, which develops the pathogenesis of many diseases.(Gloire et.al. 2006) Cellular toxicity is mediated by the ROS by reacting with cysteine residues, peroxidation of lipid, DNA damage and strand breaks. Tumor necrosis factor, lipopolysaccharide and interlukin comprises the common activators of NF-kB.(Staal et.al.1990) Hydrogen peroxide is the most common molecule generated by inflammatory signals under oxidative stress. It is reported that activation of NF-kB is inhibited by the polyphenol herbal antioxidants which was induced by minute concentration of hydrogen peroxide.(Karunaweera et.al. 2015)

METHODOLOGY

1. Protocol:



2. Materials used: Tools, databases and softwares.

NCBI: The national Cenre for BiotechnlogyInformaion(NCBI) is part of the united states National Library of Medicine(NLM), a branch of the National Insitute of Health(NLM). The NCBI creates pibluc databases, conducts research in computational biology, develops software tools for analyzing genome data and disseminates biomedical information to better understand molecular processes affecting human health.

BLAST: The Basic Local Alignment Search Tool(BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates he statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

PDB(Protein Data Bank): The Protein Data Bank(PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins, nucleic acids and complex assemblies. The Protein Data Bank file format is a textual file format describing the three-dimensional structures of molecules held in the Protein Data Bank.

Homology Modeling: Homology modeling, is known as comparative modeling of priteins, refers to constructing an atomic resolution model of the target protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein(template).

SPDBV: Swiss PDB viewer is a multi-plaform protein structure visualization program. SPDBV visualize protein surfaces and permits computation and visualization of the electrostatic potential of proteins.

ProSA: ProSA is a tool widely used to check 3D models of protein structures for potential errors. The overall quality score calculates by ProSA for a specific input structure is displayed in a plot that shows the scores of all experimentally determined protein chains currently available in the Protein Data Bank.

PyMOL: PyMOL is a python enhanced molecular graphic tool. It excels at 3Dvisualisation of proteins, small molecules, density, surfaces and trajectories. The Py part of the sofware's name refers that it extends and is extensible by the programming language python.

PubChem: Pubchem is a database of chemical molecules and their acivities against biological assays. PubChem is a massive open repository of experimental data that is organized in three distinct databases: PubChem substance, PubChem compound, PubChem bioassay.

ChemSketch: ChemSketch is a molecular modeling program used to create and modify images of chemical structures. ChemSketch allows to draw chemical structures including organics, organometallics, polymers and markush structures.

AutoDock Vina: AutoDock Vina is an open source program for doing molecular docking. AutoDock Vina improves the average accuracy of the binding mode predictions. For its input and output, AutoDock Vina uses the PDBQT molecular structure file format. AutoDock Vina generate the predicted binding modes and affinities of a ligand in seconds to minutes, depending on the ligand complexity.

Cmd: Command prompt, also known as cmd.exe, is the command line interpreter on windows.

Discovery Studio: Discovery Sudio is a protein modeling program that contains tools to visualize, analyse, modify and simulate protein structures.

3. **Disease selection:** First, we collected molecular genetic information related to oxidative stress and NF-kB p-100 subunit by reviewing the literature.

4. **Target selection and its modeling:**
   - Then target (NF-kB p-100) was selected and its sequence was retrieved and then protein homology modeling was done by using modellor to perform further studies.
   - From the generated models the best one was selected based on its C-score.
   - Energy minimization was done using SPDBV (Swiss PDB Viewer)to repair the distorted geometries and was analyzed by comparing the residues in the ramachandran plot.

5. **Drug selection:**
   - We identified4polyphenolic herbal anticancer drugs and then studied its various parameters to understand its pharmacological properties.(Quercetin, Theaflavin, Piperine and Myrecetin)
   - The drug Silymarin (polyphenol) which is isolated from plant *Milk Thistle* was taken as the test drug against the common used herbal extracts. Silymarin has high antioxidant property and is commonly used as liver detoxifier.

6. **Parameters study of drugs:**
   - For parameters study we used pubchem to know about its molecular weight, log p, IUPAC name, mol. Mass, volume etc.
   - Molecular properties and drug likeness were analyzed using Molsoft web tool.

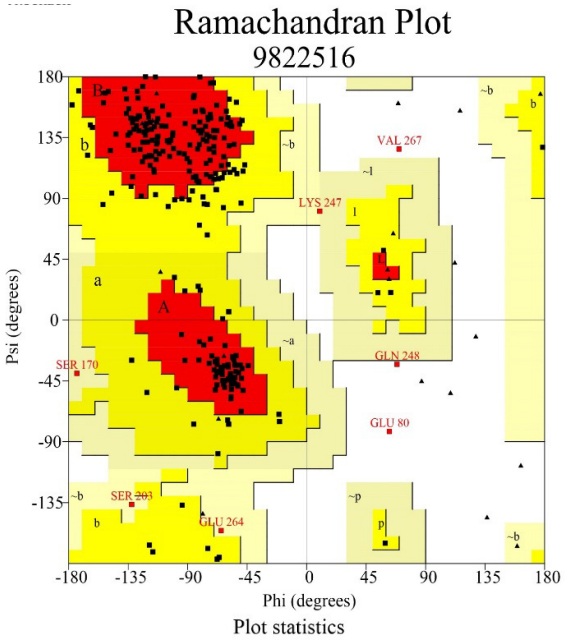
- Mutagenic, tumorigenic, reproductive effect and irritant properties of the drugs were analyzed in Datawarrior software using their canonical smiles.

7. **Selection of best inhibitor:** On analyzing all the parameters of drug likeness, mutagenic, tumorigenic, reproductive effect and irritant properties of all the drug molecules, Silymarin stands out to be the best based on highest drug likeness score and with zero side effects.

8. **Docking:** The drug and protein was docked with the help of advanced and automated molecular drug docking server called autodock vina in order to perform the complete binding affinities between the modeled protein and the drug.
   - Active site of the generated model was predicted by active site predictor tool of SCFbio.
   - 10 models were generated with different possible interactions of the protein and ligand.
   - We evaluated the docking results for binding affinity using molecular tools using Discovery Studio Software

RESULTS

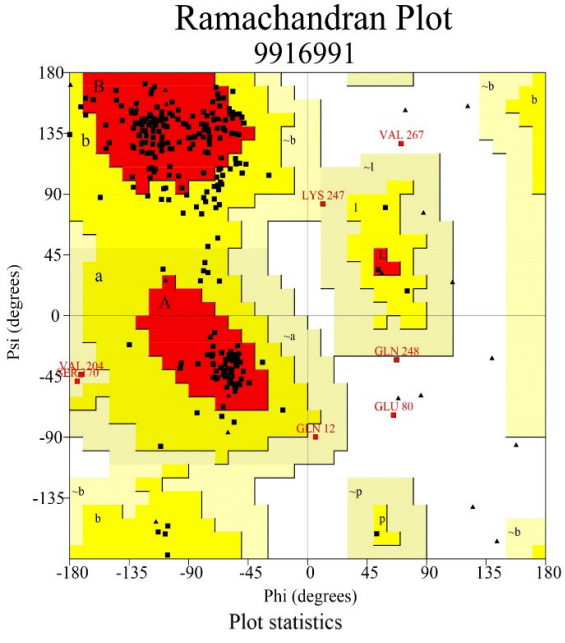
1. The geometric distortion was repaired by energy minimization and was analyzed by the translocation of the amino acid residues towards the allowed region after 80% energy minimization in the Ramachandran Plot.



Plot statistics

Residues in most favoured regions [A,B,L]	187	75.1%
Residues in additional allowed regions [a,b,l,p]	55	22.1%
Residues in generously allowed regions [~a,~b,~l,~p]	4	1.6%
Residues in disallowed regions	3	1.2%
Number of non-glycine and non-proline residues	249	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	24	
Number of proline residues	19	
Total number of residues	293	

Figure 2(a): Original Plot

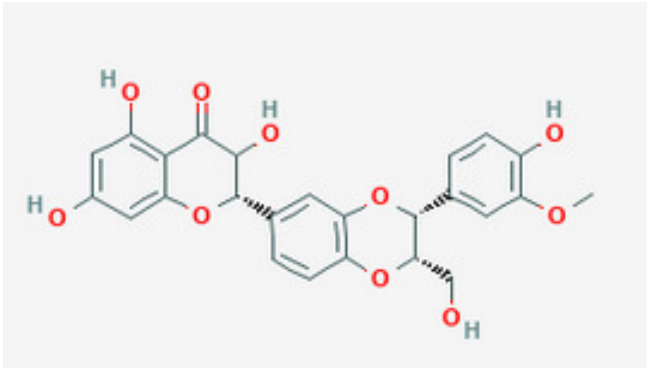


Plot statistics

Residues in most favoured regions [A,B,L]	186	74.7%
Residues in additional allowed regions [a,b,l,p]	56	22.5%
Residues in generously allowed regions [~a,~b,~l,~p]	4	1.6%
Residues in disallowed regions	3	1.2%
Number of non-glycine and non-proline residues	249	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	24	
Number of proline residues	19	
Total number of residues	293	

Figure 2(b): After 80% energy minimisation

2. Using PubChem database various parameters like structure, IUPAC name, molecular formula, molecular weight and the drug information were retrieved.



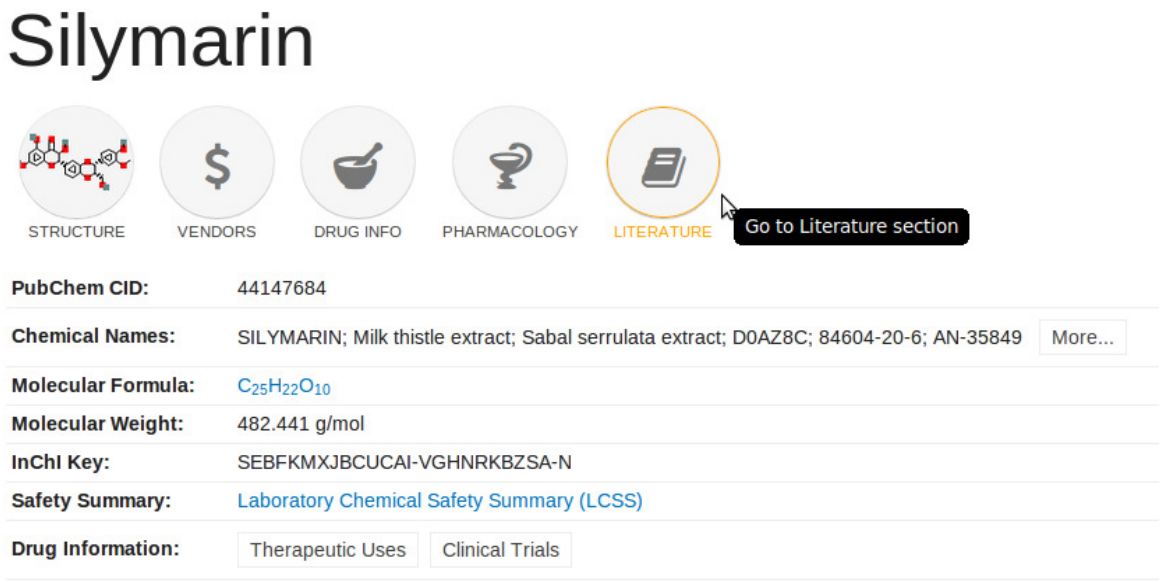
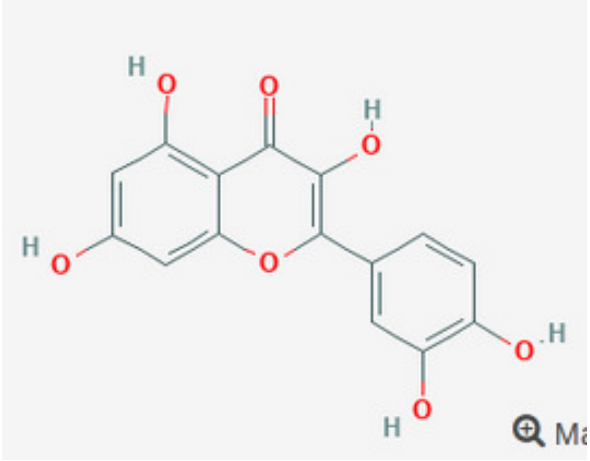


Figure 3: Pubchem result of Silymarin displaying its chemical properties.Silymarin is a flavonolignan isolated from milk thistle, *Silybummarianum*, that has been shown to exhibit antioxidant activity.



PubChem Quercetin (Compound)

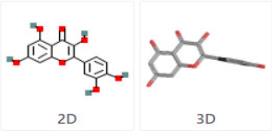
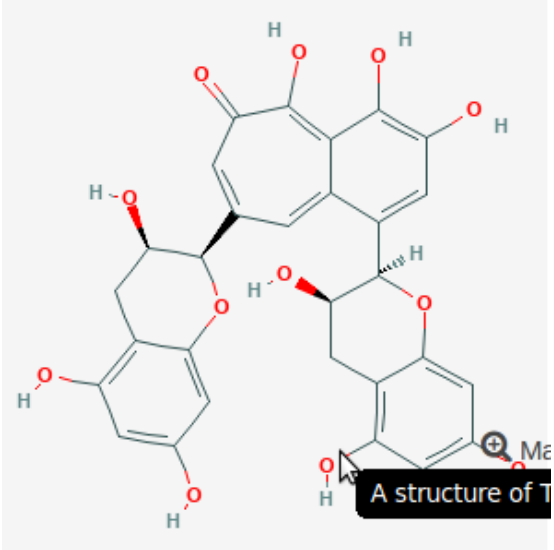
PubChem CID:	5280343
Structure:	 2D 3D Find Similar Structures
InChI Key:	REFJWTPEDVJJIY-UHFFFAOYSA-N
Molecular Formula:	$C_{15}H_{10}O_7$
UNII:	9IKM0I5T1E
Chemical Names:	quercetin 117-39-5 Meletin Sophoretin Quercetine More...
Molecular Weight:	302.238 g/mol
Dates:	Modify: 2019-04-20 Create: 2004-09-16

Figure 4: Pubchem result of Quercetin displaying its chemical parameters. Quercetin, widely distributed in plant food sources and a major bioflavonoid in the human diet, may produce antiproliferative effects by the modification of NF-kB pathway.

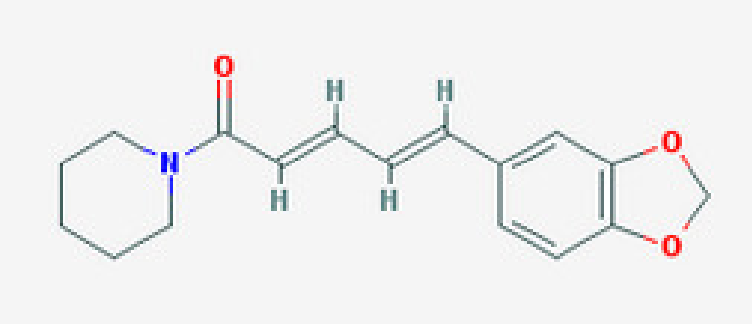


Theaflavin

STRUCTURE VENDORS PHARMACOLOGY LITERATURE PATENTS BIOACTIVITIES

PubChem CID:	114777
Chemical Names:	Theaflavin; Theaflavine; 4670-05-7; (-)-Theaflavin; UNII-1IA46M0D13; 1IA46M0D13 More...
Molecular Formula:	$C_{29}H_{24}O_{12}$
Molecular Weight:	564.499 g/mol
InChI Key:	IPMYMEWFZKHGAX-ZKSIBHASSA-N
Substance Registry:	FDA UNII

Figure 5: Pubchem result of Theaflavin showing its various chemical properties. Theaflavin is a class of natural flavonoids derived from the dried leaves of the plant *Camellia sinensis* (tea) and related plants with potent antioxidant properties.

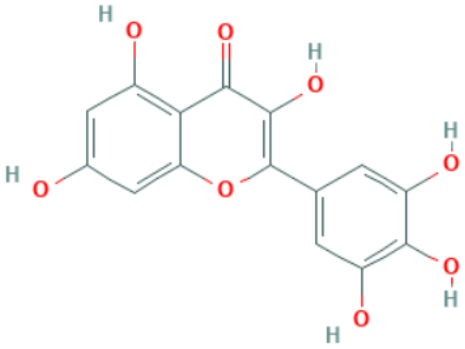


Piperine

STRUCTURE VENDORS DRUG INFO PHARMACOLOGY LITERATURE PATENTS BIOACTIVITIES

PubChem CID:	638024
Chemical Names:	Piperine; 94-62-2; 1-Piperoylpiperidine; Piperin; Bioperine; Piperoylpiperidine More...
Molecular Formula:	$C_{17}H_{19}NO_3$
Molecular Weight:	285.343 g/mol
InChI Key:	MXXWOMGUGJBKIW-YPCIICBESA-N
Drug Information:	Clinical Trials FDA UNII
Safety Summary:	Laboratory Chemical Safety Summary (LCSS)

Figure 6: Pubchem result of Piperine showing its various chemical properties. It is an alkaloid isolated from the plant *Piper nigrum*. It has a role as a NF-kappaB inhibitor, a plant metabolite, a food component and a human blood serum metabolite.



Myricetin

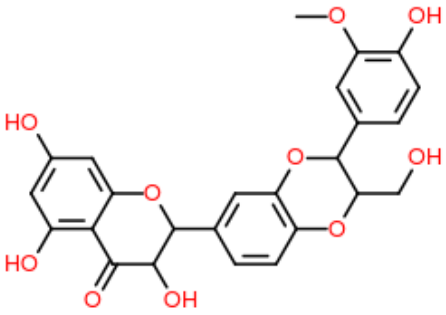
STRUCTURE VENDORS PHARMACOLOGY LITERATURE PATENTS BIOACTIVITIES

PubChem CID:	5281672
Chemical Names:	Myricetin; 529-44-2; Cannabiscetin; Myricetol; Myricitin; 3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one More...
Molecular Formula:	$C_{15}H_{10}O_8$
Molecular Weight:	318.237 g/mol
InChI Key:	IKMDFBPHZNJCSN-UHFFFAOYSA-N
Substance Registry:	FDA UNII
Safety Summary:	Laboratory Chemical Safety Summary (LCSS)

Figure 7: Pubchem result of Myricetin showing its various chemical properties. It has been isolated from the leaves of *Myrica rubra* and other plants. It has a role as a NF-kB canonical pathway inhibitor, an antineoplastic agent and an antioxidant agent.

3. The drug likeness score was analyzed by molsoft web tool. The model score was generated by fragmenting the molecule and aligning them in random linear and non-linear manner and counting the occurrence value for water solubility, partial coefficient and polar surface area by root mean square prediction. From the database regression model the model score of 0.92 and above is considered as best score.

Molecular Properties and Drug-likeness.



Molecular formula: C25 H22 O10
Molecular weight: 482.12
Number of HBA: 10
Number of HBD: 5
MolLogP : 2.59
MolLogS : -6.19 (in Log(moles/L)) 0.31 (in mg/L)
MolPSA : 126.94 A^2
MolVol : 439.51 A^3
Number of stereo centers: 4

Drug-likeness model score: 0.99

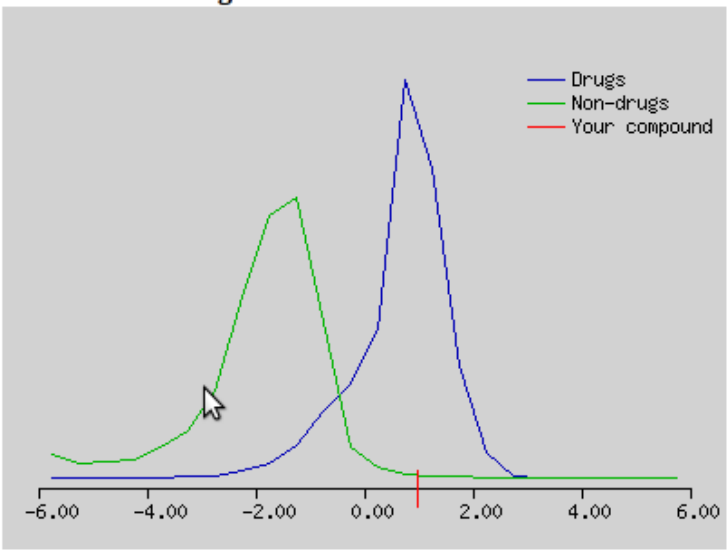
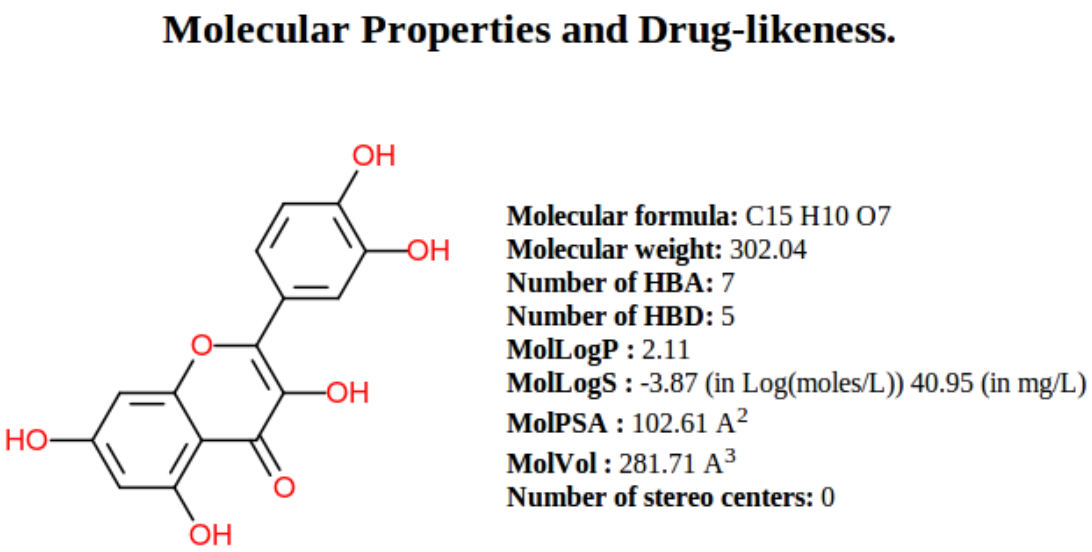


Figure 8: Molsoft result of Silymarin showing highest drug likeness model score of 0.99 and its standard molecular properties.



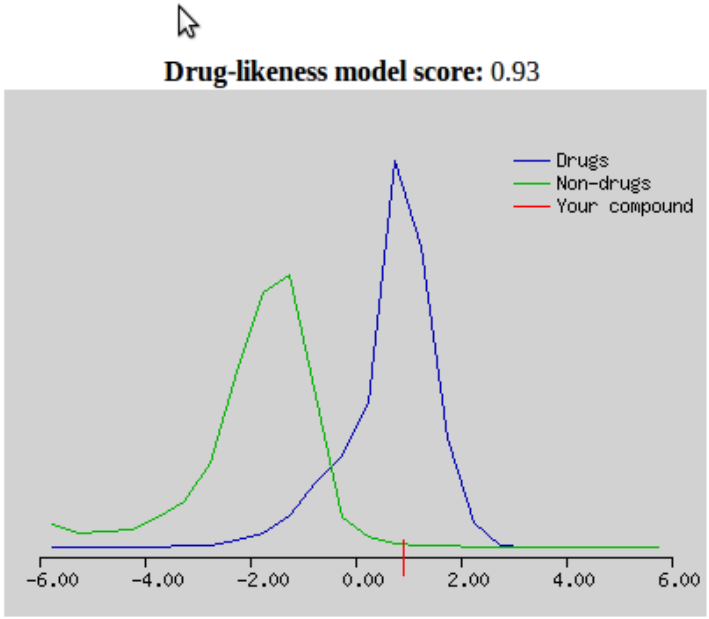
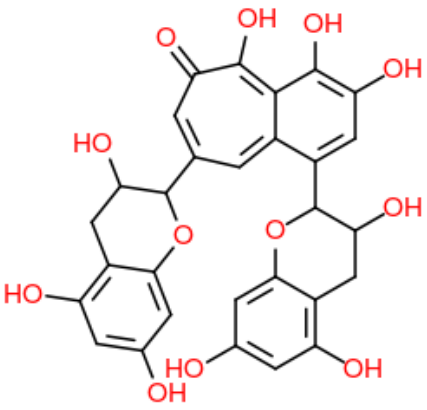


Figure 9: Molsoft result of Quercetin showing drug likeness score of 0.93.

- The model was rejected due to less polar surface area as compared to silymarin.

Molecular Properties and Drug-likeness.



Molecular formula: C29 H24 O12
Molecular weight: 564.13 (> 500)
Number of HBA: 12 (> 10)
Number of HBD: 9 (> 5)
MolLogP : 2.14
MolLogS : -5.21 (in Log(moles/L)) 3.45 (in mg/L)
MolPSA : 175.06 A^2
MolVol : 542.84 A^3
Number of stereo centers: 4

Drug-likeness model score: 0.31

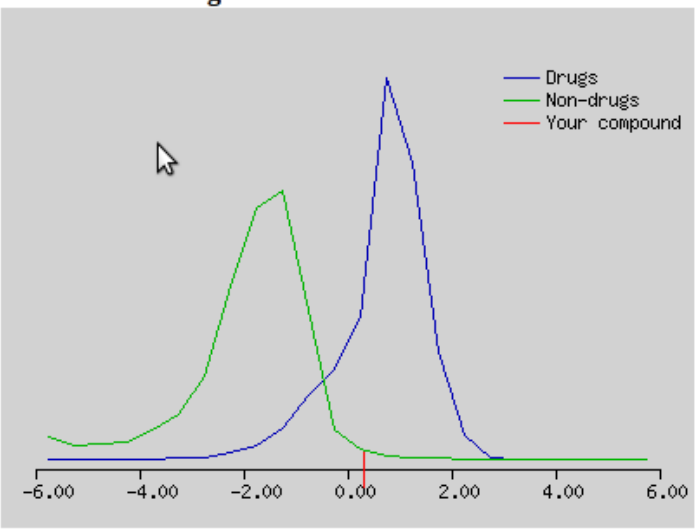
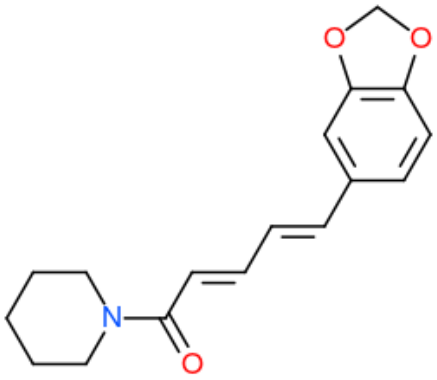


Figure 10: Molsoft result of Theaflavin showing the drug likeness score of 0.31.

- Low likeness score was due to high molecular weight and more number of hydrogen bond donor and acceptors making it highly reactive and unstable.

Molecular Properties and Drug-likeness.



Molecular formula: C17 H19 N O3
Molecular weight: 285.14
Number of HBA: 3
Number of HBD: 0
MolLogP : 3.96
MolLogS : -4.88 (in Log(moles/L)) 3.77 (in mg/L)
MolPSA : 33.47 A^2
MolVol : 328.92 A^3
Number of stereo centers: 0

Drug-likeness model score: -0.02

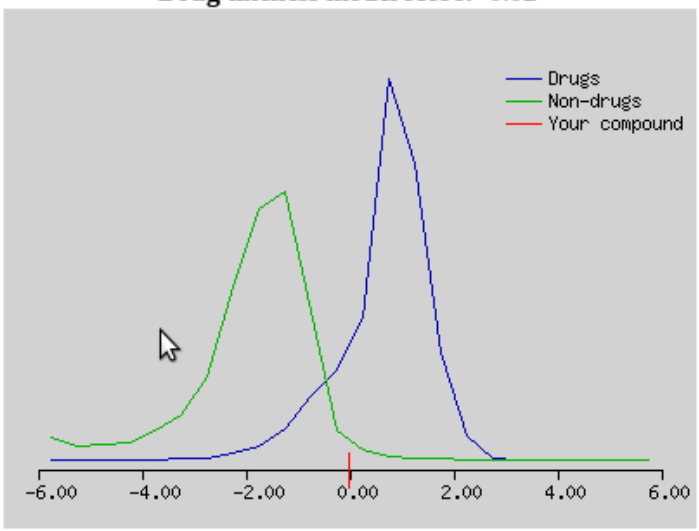
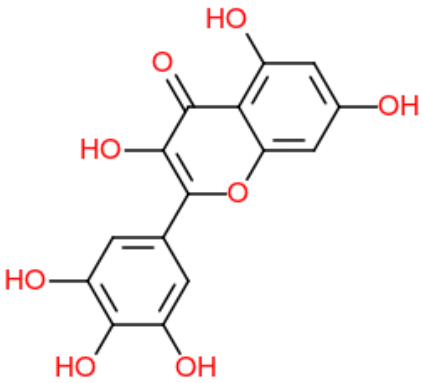


Figure 11: Molsoft result of Piperine showing negative likeness score of -0.02.

- It is due to the negative water solubility (LogS value).

Molecular Properties and Drug-likeness.



Molecular formula: C15 H10 O8
Molecular weight: 318.04
Number of HBA: 8
Number of HBD: 6 (> 5)
MolLogP : 1.13
MolLogS : -3.29 (in Log(moles/L)) 164.52 (in mg/L)
MolPSA : 118.09 A^2
MolVol : 292.39 A^3
Number of stereo centers: 0

Drug-likeness model score: -0.04

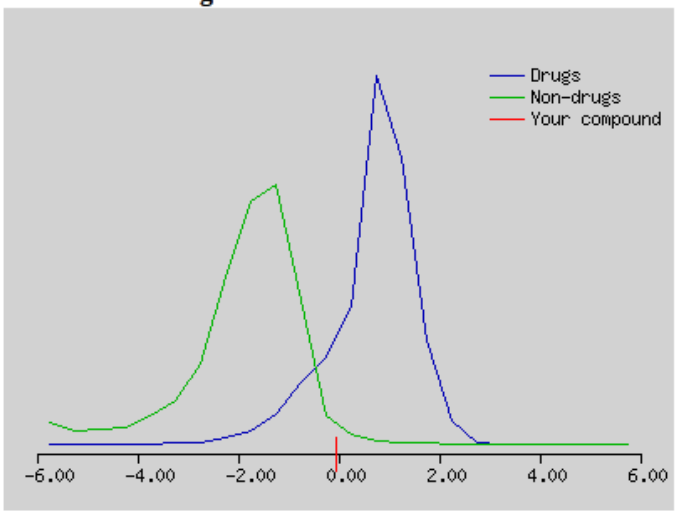


Figure 12: Molsoft result of Myricetin showing drug likeness model score of -0.04.

- It is due to high number of hydrogen bond donors and negative water solubility.

4. Physicochemical properties like mutagenic, tumorigenic, reproductive effect and irritant properties were analyzed in datawarrior software using canonical smiles.

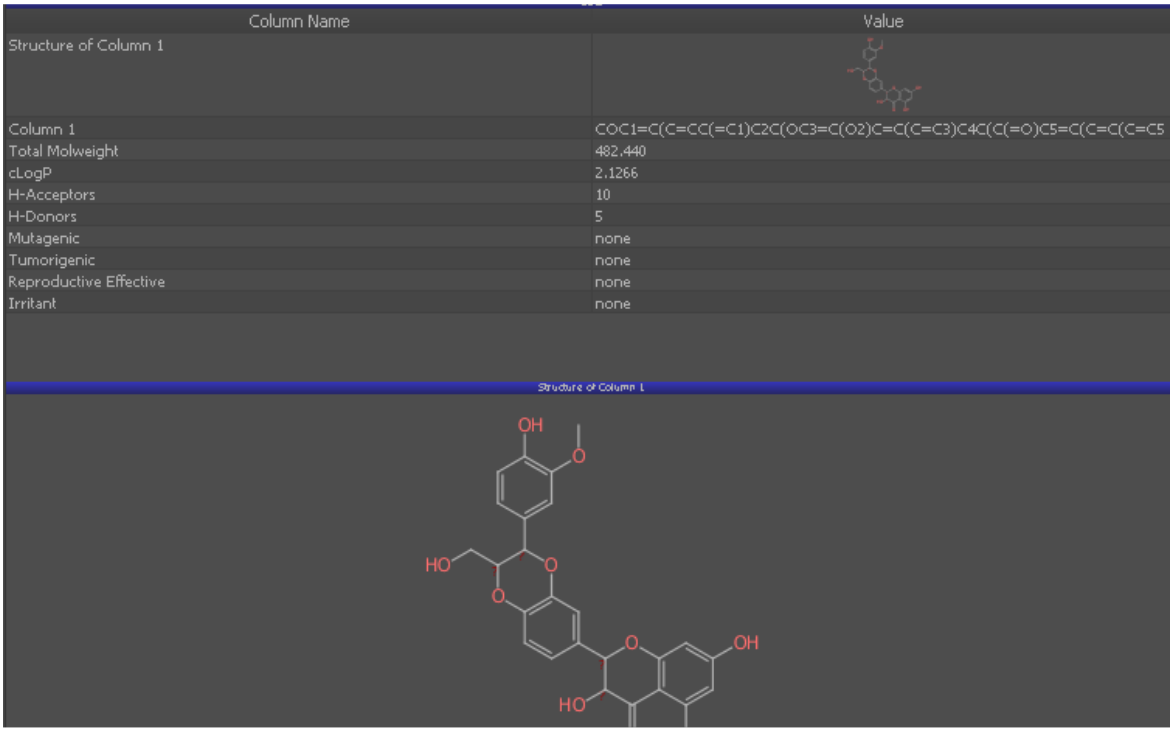


Figure 13: Datawarrior result of Silymarin shows no physicochemical side effects.

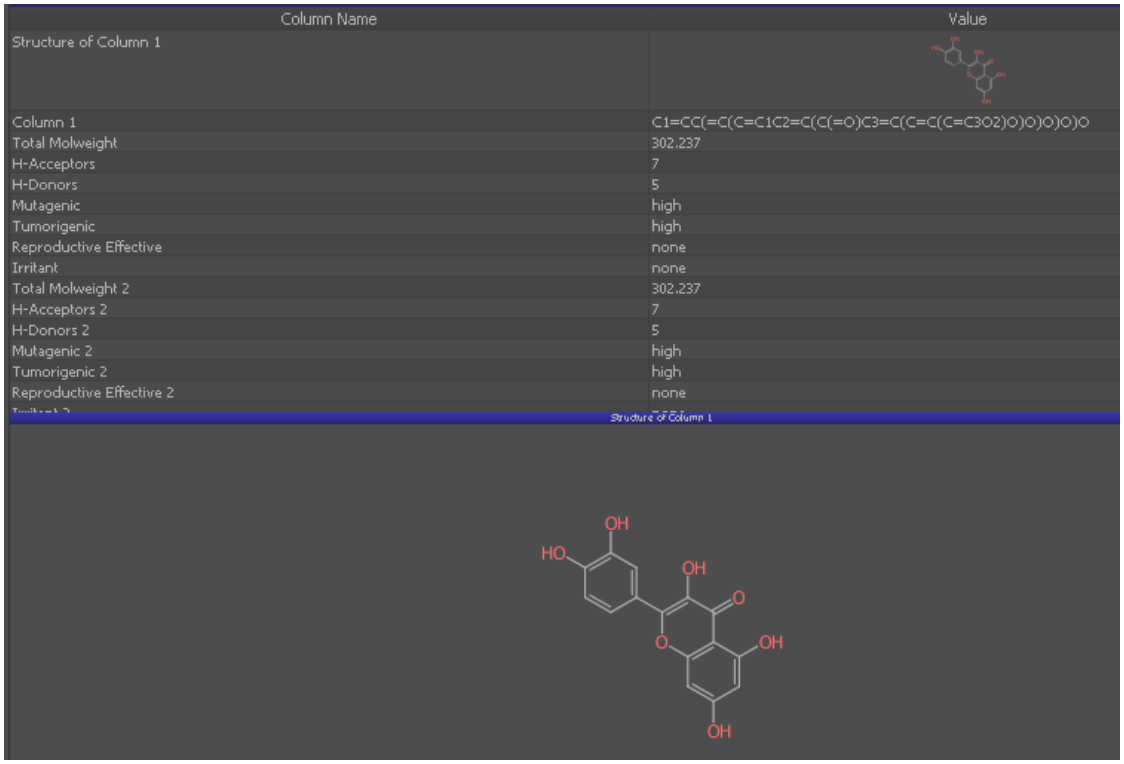


Figure 14: Datawarrior result of Quercetin shows high mutagenic and tumorigenic effect.

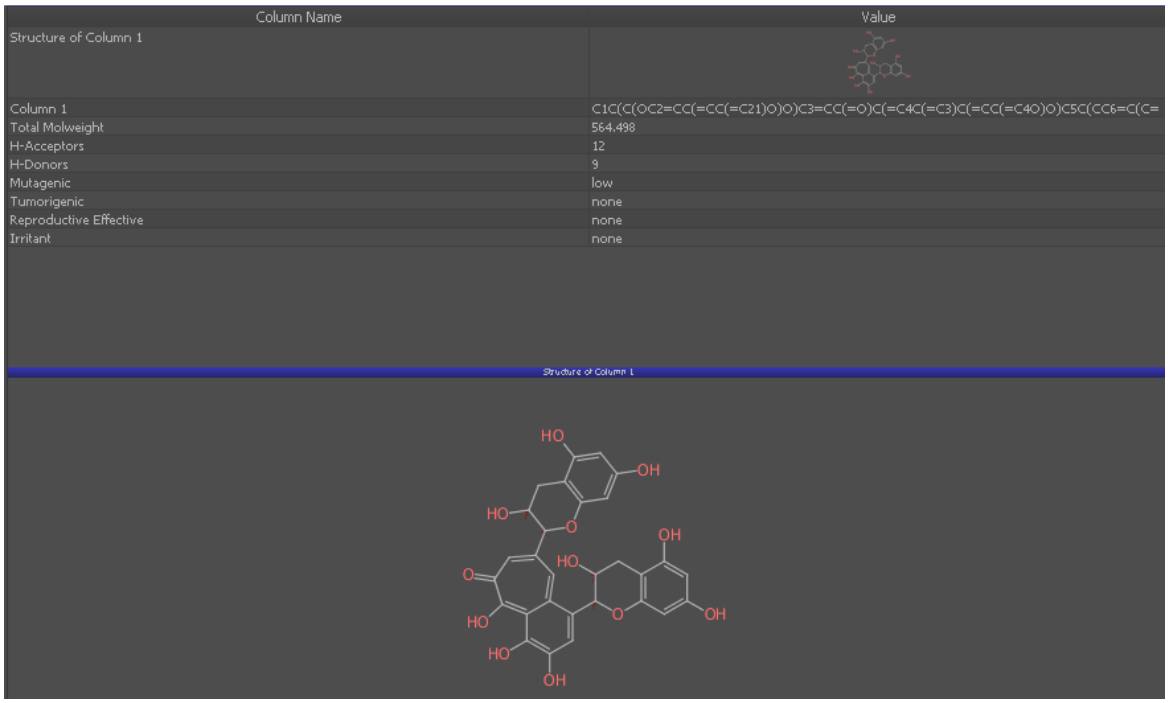


Figure 15: Datawarrior result of Theaflavin shows low mutagenic effect.

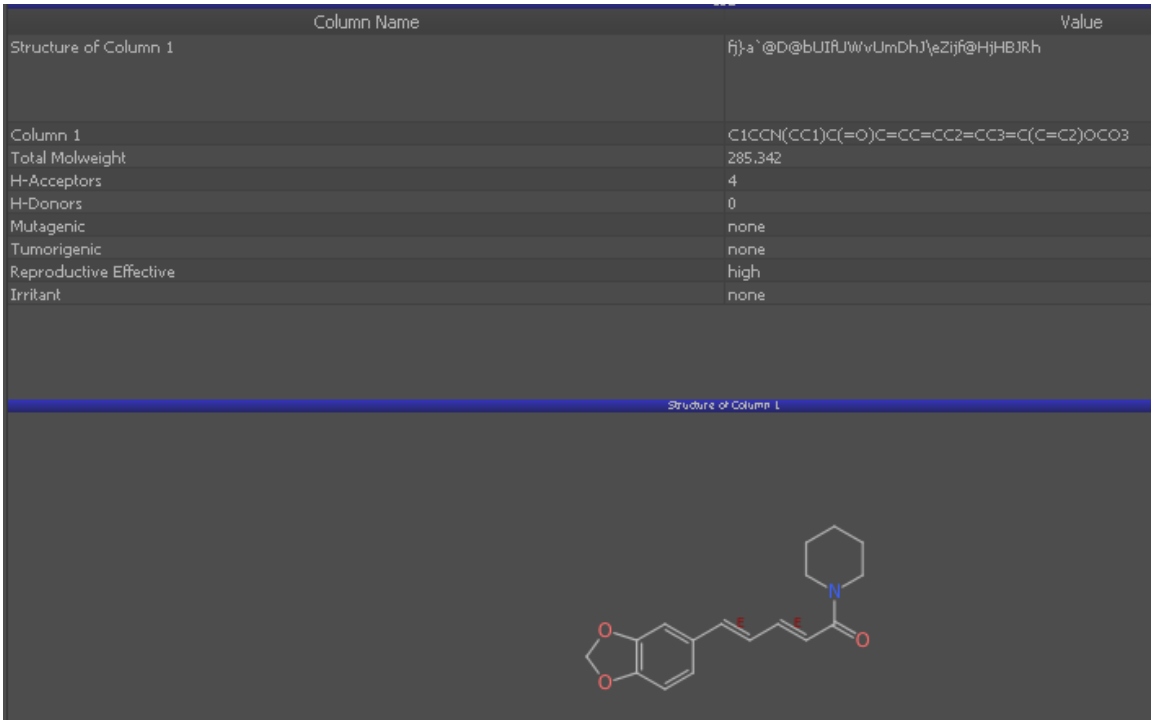


Figure 16: Datawarrior result of Piperine shows high reproductive effect.

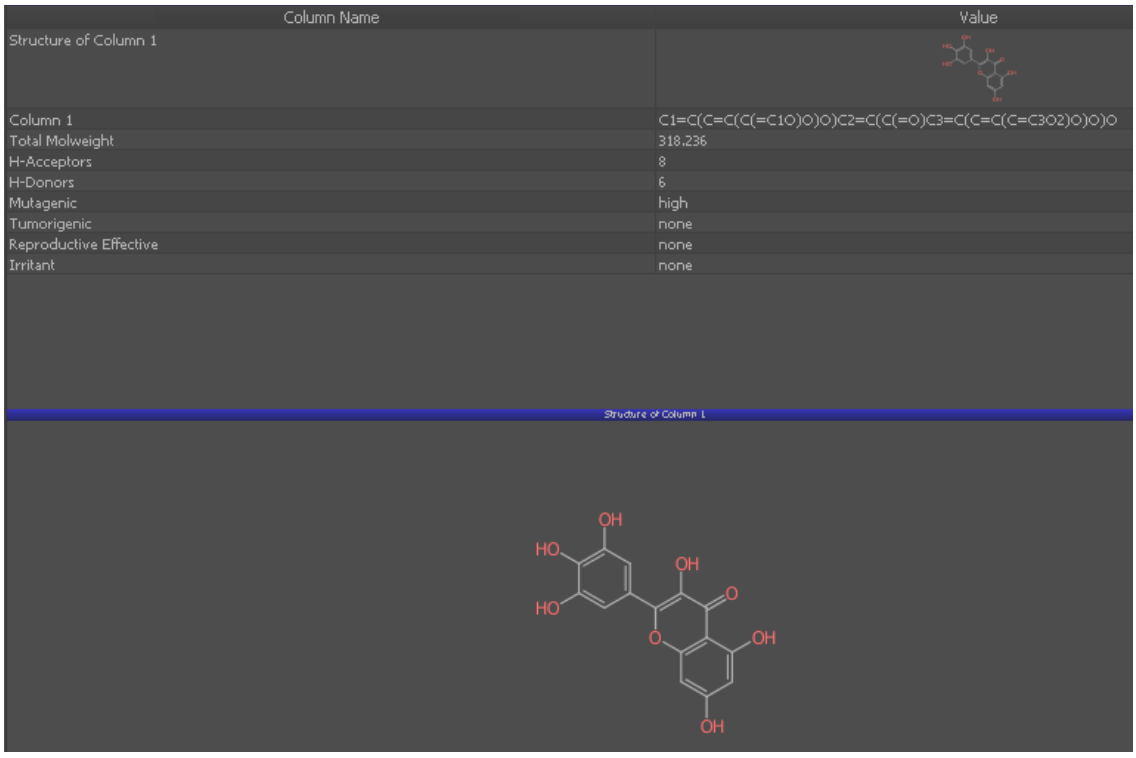


Figure 17: Datawarrior result of Myrecetin shows high mutagenic effect.

- On analyzing all the parameters obtained, Silymarin was selected to be the best inhibitor ligand to perform docking

5. Active site predictor web-tool of SCFbio was used to predict the active site.

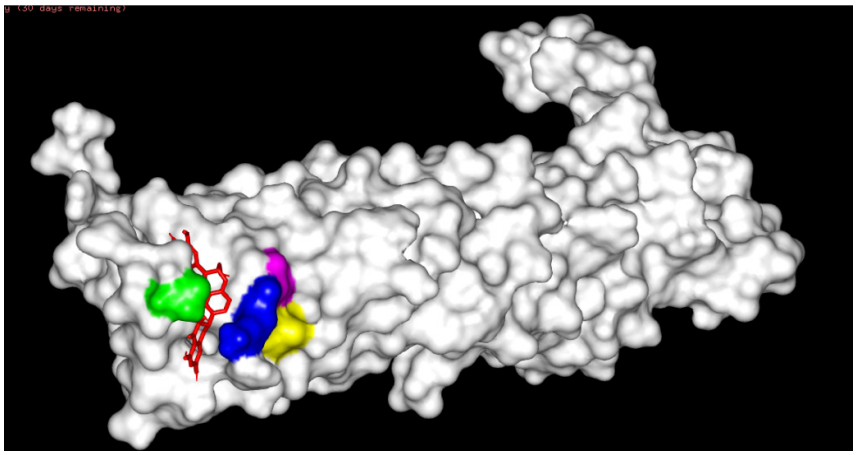


Figure 18: Predicted active site with coloured amino acid residues. (Leucine:688-Green, Arginine:685-yellow, lysine:689-pink and threonine:753-blue)

6. Discovery studio software was used to analyze the docking results for the binding affinity and the type of interactions.

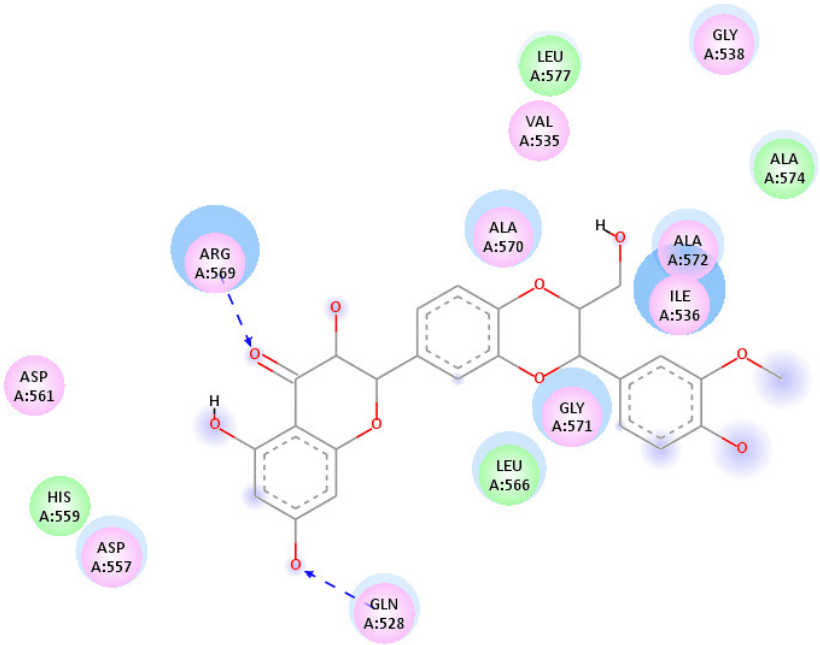


Figure 19: Model 1 (binding affinity: -7.9, Interaction: Arg569(O); Gln528(O))

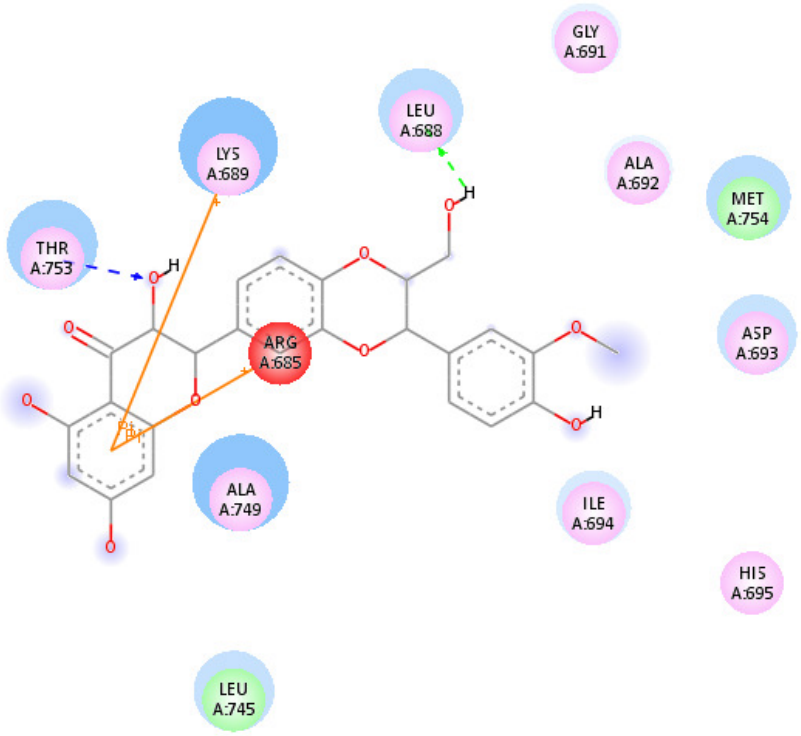


Figure 20: Model 2 (binding affinity: -7.3, Interaction: Thr753(O); Leu688(H); Arg685(Pi); Lys689(Pi))

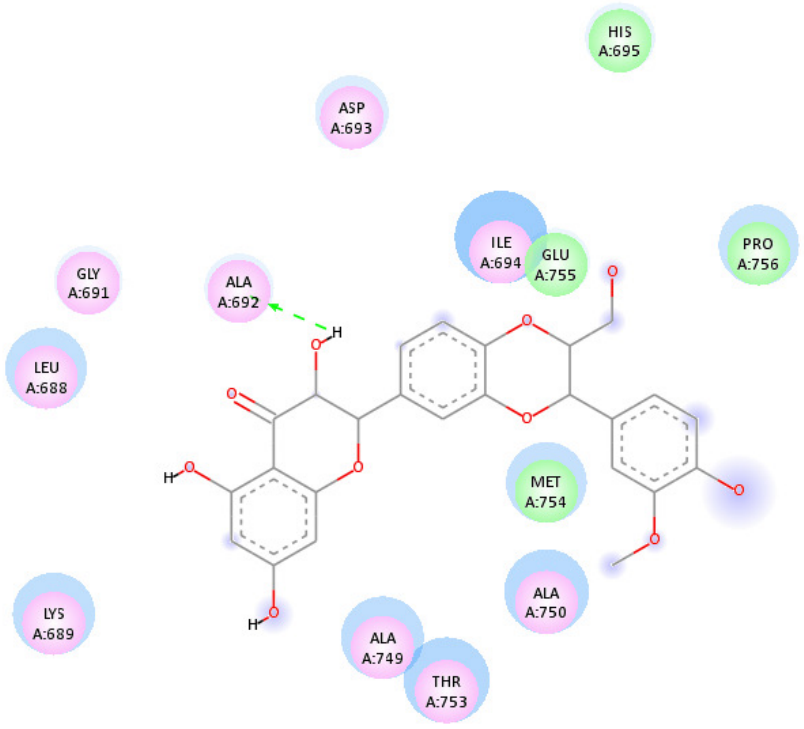


Figure 21: Model 3 (binding affinity: -7.1, Interaction: Ala692(H))

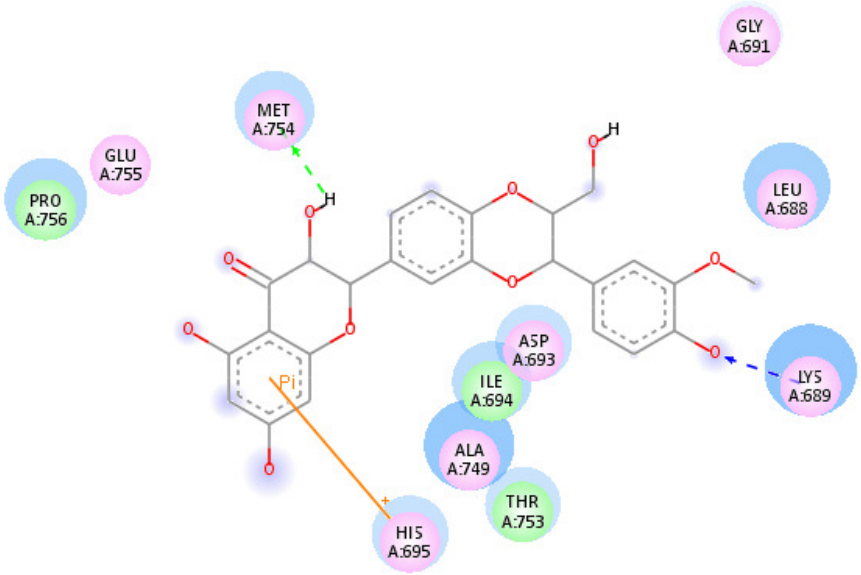


Figure 22: Model 4 (binding affinity: -7, Interaction: Met754(H); Lys689(O); His695(Pi))

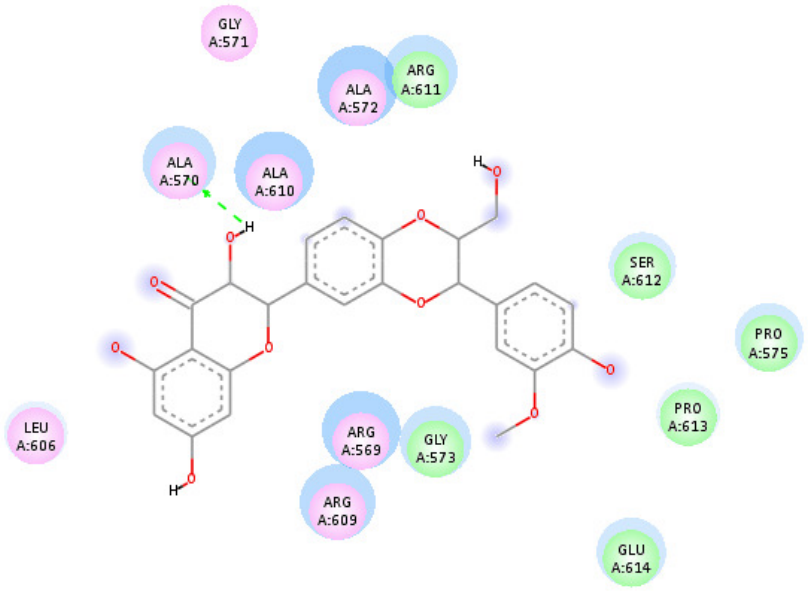


Figure 23: Model 5 (binding affinity: -7, Interaction: Ala570(H))

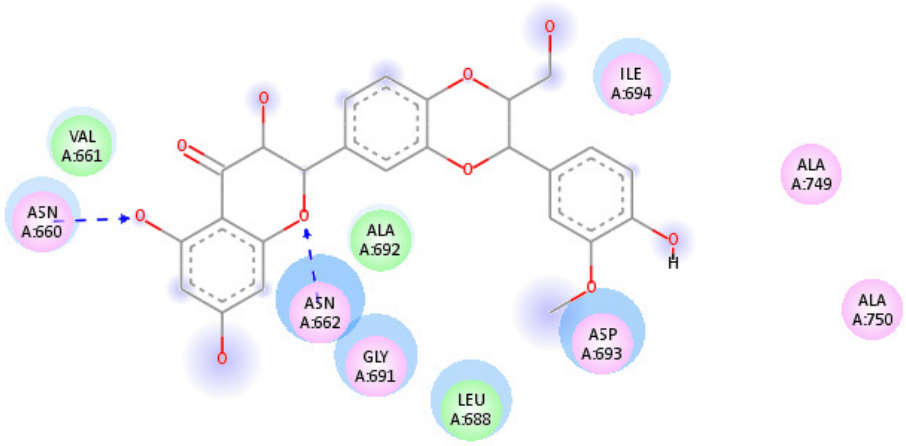


Figure 24: Model 6 (binding affinity: -6.9, Interaction: Asp693(H); Leu688(H))

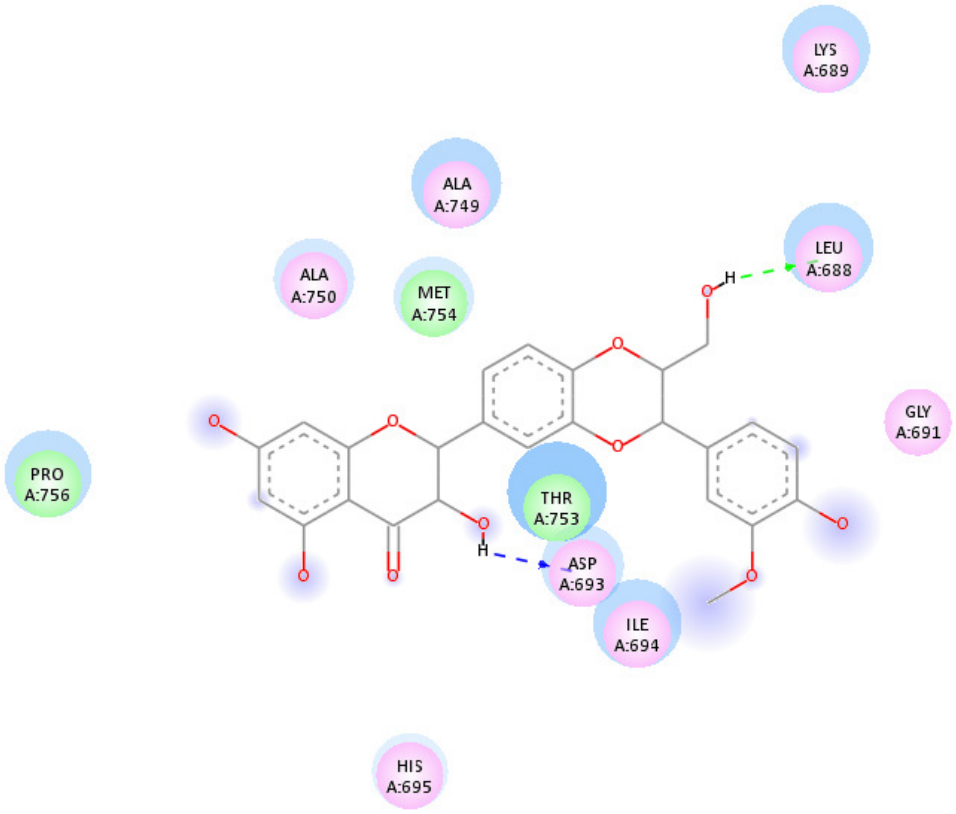


Figure 25: Model 7 (binding affinity: -6.8, Interaction: Asn660(O); Asn662(O))

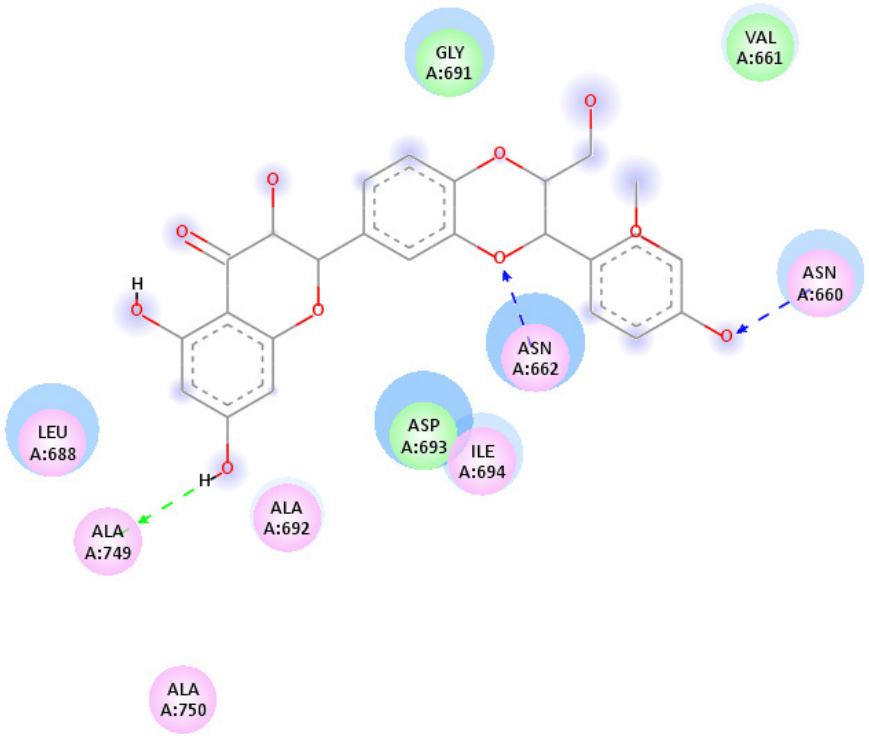


Figure 26: Model 8 (binding affinity: -6.8, Interaction: Ala749(H); Asn660(O); Asn662(O))

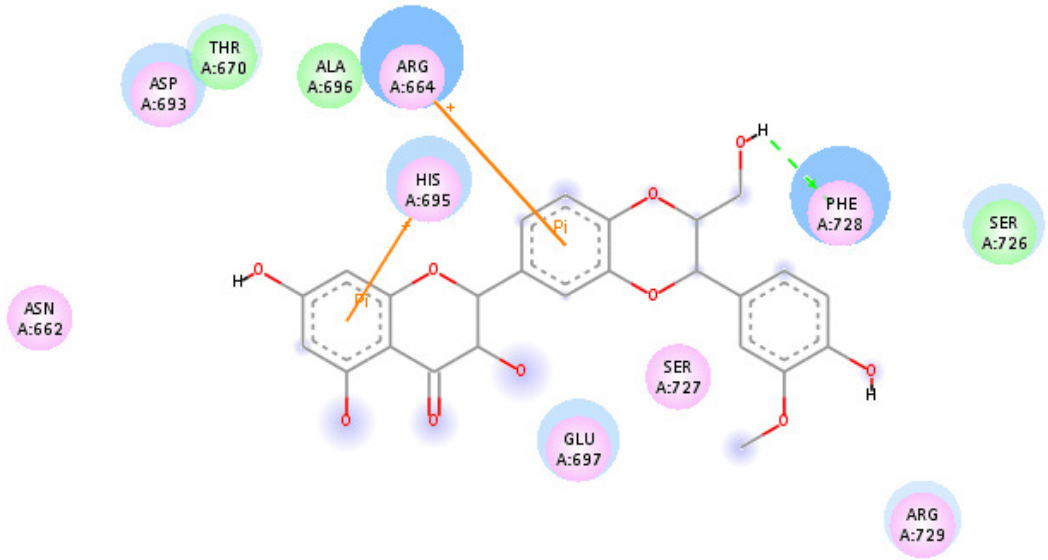


Figure 27: Model 9 (binding affinity: -6.8, Interaction: Phe728(H); Arg664(Pi); His695(Pi))

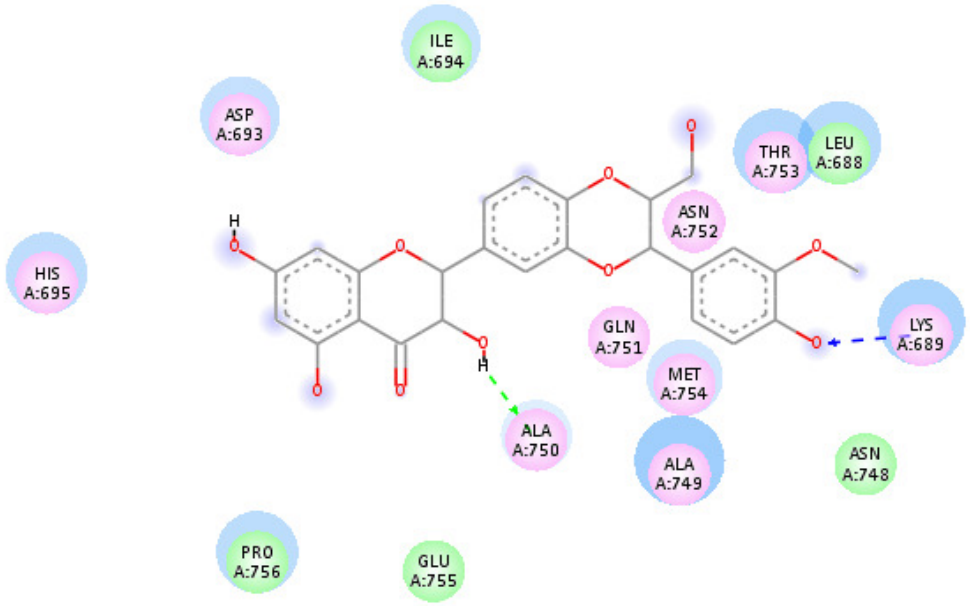


Figure 28: Model 10 (binding affinity: -6.8, Interaction: Lys689(O); Ala750(H))

Model	Binding affinity	Type of interaction
1	-7.9	Arg569(O); Gln528(O)
2	-7.3	Thr753(O); Leu688(H); Arg685(Pi); Lys689(Pi)
3	-7.1	Ala692(H)
4	-7	Met754(H); Lys689(O); His695(Pi)
5	-7	Ala570(H)
6	-6.9	Asp693(H); Leu688(H)
7	-6.8	Asn660(O); Asn662(O)
8	-6.8	Ala749(H); Asn660(O); Asn662(O)
9	-6.8	Phe728(H); Arg664(Pi); His695(Pi)
10	-6.8	Lys689(O); Ala750(H)

Table 1: Binding affinity and type of interaction of the docked models

- On analysing the docking results, model 2 had more stronger interaction with respect to higher binding affinity as more negative score.

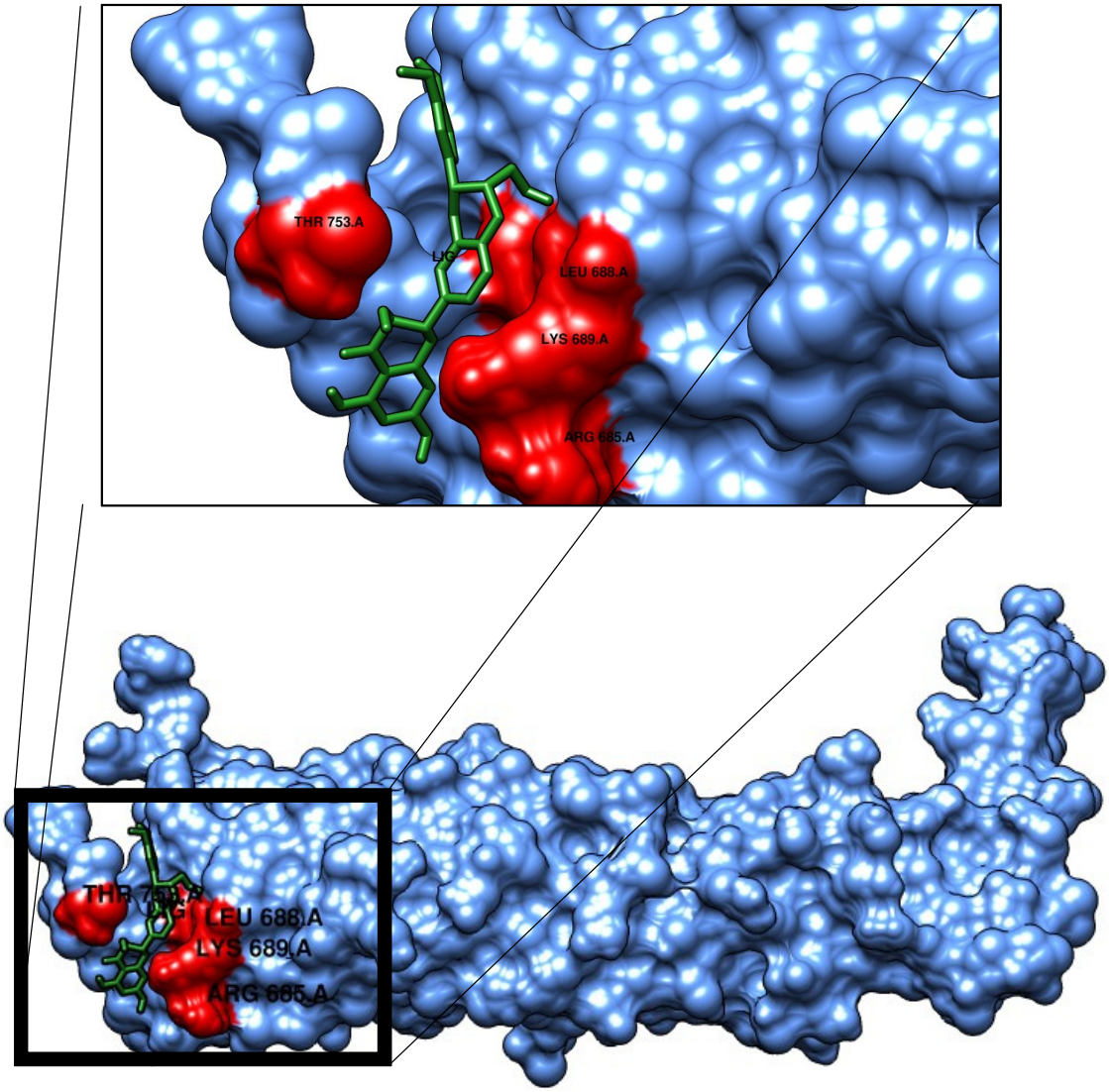


Figure 29: Ligand docked with active site residues (Leucine:688, Arginine:685, lysine:689 and threonine:753)

- The Docking was performed successfully with the active site of the generated model of the NF-kB p100 subunit.

CONCLUSION AND DISCUSSION

Oxidative stress has become a major root cause for the development of various other disease. The modern lifestyle has contributed a lot to develop this stress condition like the unhealthy diet, alcohol, smoking and even the environmental pollution due to the rage of development without looking at the consequences for future aspects.

The production of free radicals and the ROS develops a negative electrostatic potential in the body and further produces more ROS by conducting a chain reaction by oxidation of lipids and proteins. This access accumulation of ROS interferes in different metabolic pathways either by increasing or supressing the protein expression leading to biochemical imbalance in the body. The current studied NF-kB pathway is accelerated by the presence of excess ROS and inhibits the I-kB by its phosphorylation. All this process leading to uncontrolled cell proliferation further causing tumor/cancer formation.

Chemo/radio therapy is the most common treatment method for the tumor/cancer. These process only kills the new proliferated cell and doesn't stops the formation of new cells and also have very harmful side effects. Instead of chemo/radio therapy we can use herbal extracts as targeted drug compound for naturally controlling the gene expression.

Most of the herbs used as anti-cancerous drugs are projected to inhibit the classical pathway of the NF-kB i.e. p65/RELA domain. All those herbs have various side effects that may be harmful for future aspects. Even after inhibition of the classical pathway, cell proliferation can go on through the alternative pathway.

But Silymarin inhibits the alternative pathway of NF-kB by binding to p100/RELB domain which suppresses the kinase enzyme that phosphorylates the I-kB. The active I-kB helps to inhibit the classical pathway by binding to the p65/RELA domain and stops its translocation to nucleus further preventing the DNA transcription, which in turn finally stops cell proliferation.

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List of Abbreviations

ROS: Reactive oxygen species

DNA: Deoxiribo-nucleic acid

NF-kB: Nuclear Factor kappa B

Nrf2: Nuclear related factor 2

I-kB: Inhibitory kappa B

IKK: Inhibitory kB kinase

NIK: NF-kB inducing kinase

NEMO: NF-kB essential modulator

