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MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATION OF PHYTOCHEMICAL DERIVED COMPOUNDS AS POTENTIAL ANTI CANCER AGENT AGAINST TYROSINE KINASE

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ABSTRACT

There is a continuous requirement to develop novel, safe, effective and affordable anti cancer drugs because cancer is a serious at current situation. A huge number of patients die annually due to cancer disease. Phytochemical are the secondary metabolites of medicinal plants and significantly used in conventional cancer research. Bioactive phytochemical are favored as they claim differentially on cancer cell only without altering normal cell. Carcinogenesis is an intricate process and includes multifold signaling procedures. Phytochemical are pleiotropic in nature, function and target these events in multiple manners so they are considered as most appropriate candidate for drug development. The aim of the present research was to find out the anti cancer activity of the phytochemical constituents through computer aided drug design approach. In this experiment, we have find total 42 natural compounds with anti cancer activity against the cancer target 1QCF tyrosine kinase. The data set comprising of phytochemical compounds were used for virtual screening and molecular docking in PyRx software. Along with screened compound, hit compound Cytisine was further docked to confirm the binding mode and confirmed the effective inhibition of 1QCF and anticancer activity. Molecular dynamic simulation studies were done to confirm the stability of the protein and ligand complex during a simulation. Parameters like RMSD, RMSF, and radius of gyration were experiential to understand the fluctuations. Protein-ligand interaction studies also expose that enough hydrogen and hydrophobic bonds are present to validate our results. Our study suggests that the potential use of Cytisine can come out as a potential candidate and in turn prevent cancer.

KEYWORDS: Phytochemicals, anticancer, tyrosine kinase, virtual screening, molecular docking, molecular dynamic simulation.

1. INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. There are over 200 types of cancer.[1] These abnormal cells are termed cancer cells, malignant cells, or tumor cells. Most cancers form tumors, but not all tumors are cancerous. A tumor is a mass composed of a cluster of such abnormal cells. Benign, or noncancerous, tumors do not spread to other parts of the body, and do not create new tumors. Malignant, or cancerous, tumors crowd out healthy cells, interfere with body functions, and draw nutrients from body tissues.[2] The incidence of cancer and cancer types are influenced by many factors such as age, gender, race, local environmental factors, diet, and genetics. According to estimates from the International Agency for Research on Cancer (IARC), in 2018 there were 17.0 million new cancer cases and 9.5 million cancer deaths worldwide. By 2040, the global burden is expected to grow to 27.5 million new cancer cases and 16.3 million cancer deaths simply due to the growth and aging of the.[3] IARC estimates that globally, 1 in 5 people develop cancer during their lifetime, and 1 in 8 men and 1 in 11 women die from the disease. These new estimates suggest that more than 50 million people are living within five years of a past cancer diagnosis.[4] Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. Around one-third of deaths from cancer are due to tobacco use, high body mass index, alcohol use, low fruit and vegetable intake, and lack of physical activity. Tobacco use is the most important risk factor for cancer and is responsible for approximately 25% of cancer deaths. Cancer-causing infections, such as hepatitis and human papillomavirus (HPV), are responsible for approximately 30% of cancer cases in low- and lower-middle-income countries. Cancer mortality can be reduced if cases are detected and treated early. A correct cancer diagnosis is essential for appropriate and effective treatment because every cancer type requires a specific treatment regimen. Treatment usually includes radiotherapy, chemotherapy and/or surgery.[5] Cancer treatments may have many side

effects. A side effects occur when treatment damages healthy cells. Side effects can be different for each person, and for different medicines and kinds of treatment. Side effects includes Neutropenia, Lymphedema, Hair loss, Nausea, vomiting, tiredness, and depression.[6] Therefore, the focus is on the use of alternative treatment against cancer. Herbal medicines have been used in many developing countries as primary source of medical.[7] Many plant species are already used to prevent or treat cancer. The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities. Among them, about 3,000 plant species have demonstrated reproducible anticancer activity.[8] India is the largest producer of medicinal plants and is as the "Botanical garden of the World". The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. Considerable works have been done on these plants to treat cancer, and some plant products have been marketed as anticancer drugs, based on the traditional uses and scientific reports.[9] The process of discovery and development of novel drugs is known to be time-consuming and expensive.[10] Thus, novel drug development strategies with a reduced cost of time and money, as well as an enhanced efficiency are in high demand, which would contribute to a significant improvement in global health and life expectancy. Computational methods have served as an essential tool in drug discovery projects and have been a cornerstone for new drug development approaches.[11] One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor. Docking is the process by which two molecules fit together in 3D space.[12] In these research, we are identified natural phytoconstituents using molecular docking for the treatment of cancer. By studying the literature of natural bioactive compounds we found that there are 51 natural compounds which shows anticancer activity.

Role of receptor tyrosine kinases in cancer pathway

Receptor tyrosine kinases (RTKs) are a family of cell surface receptors, which act as receptors for growth factors, hormones, cytokines, neurotrophic factors and other extracellular signaling molecules. RTKs intervene key signaling pathways that are involved in cell proliferation, differentiation, survival and cell migration.[13] The RTK family consists numerous subfamilies which contain, among others, epidermal growth factor receptors (EGFRs), fibroblast growth factor receptors (FGFRs), insulin and insulin-like growth factor receptors (IR and IGFR), platelet-derived growth factor receptors (PDGFRs), vascular endothelial growth factor receptors (VEGFRs), hepatocyte growth factor receptors (HGFRs), and proto-oncogene c-KIT.[14-15] RTKs monomers are structured into an extracellular (N-terminal), a trans membrane and a cytoplasmic kinase field.[16] They are activated via ligand-induced dimerization that results in receptor auto-phosphorylation and tyrosine activation of RTKs' substrates including phospholipase C-γ, mitogen-activated protein kinases and phosphatidylinositol 3-kinase. Mutations that affect RTK signaling often lead to cell transformation, which is observed in a wide variety of malignancies. These mutations affect RTKs or components of downstream pathways such as MAP kinase and the PI3K/AKT. This results in increased cell proliferation, survival, invasion and metastasis. Therefore, targeting RTK signaling pathways remains a challenge for scientists and clinicians working in the cancer field. Several small molecule inhibitors and antibodies are being clinically developed to target RTKs, the MAP kinase and PI3K/AKT pathways. This review attempts to highlight the important role played by RTK signaling in carcinogenesis and the therapeutic strategies available, so far, to target these important cellular pathways.

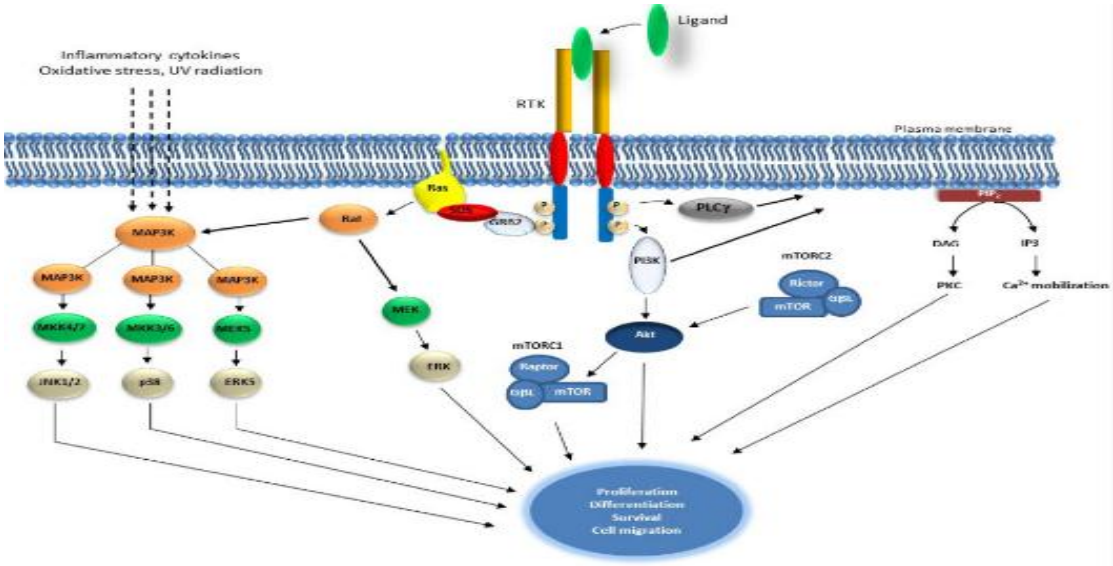


Figure 1: Schematic representation of Receptor Tyrosine Kinase and downstream signaling pathways.

2. MATERIAL AND METHODS

Protein structure preparation

The X-ray diffraction-based crystal structure of tyrosine kinase (1 QCF) with a resolution of 2.00 Å was taken from the protein data bank. The structure was cleaned to ensure maximum quality and reliability.[17] The bound ligands, water molecules were removed and missing atoms and residues were added. Stearic clashes were minimized and hydrogen atoms were added. Formal bond orders were determined, side chains were optimized and fixed, charges added using program implemented in chimera, SWISS PDB viewer, and Chiron minimization and refinement tool.[18-20]

Ligand archive research

A deep literature survey was performed to discover the natural compounds having said anticancer properties. A total of 42 compounds were compounds were identified (table 1) and their 3D structures were generated by Galaxy 3D generator tool of online server Molinspiration Chemoinformatics (https://www.molinspiration.com/) (Figure 2) and 3D structure of target protein tyrosine kinase (PDB ID: 1QCF). The 3D structures were visualized by Discovery Studio Visualizer. The compounds were imported in to the PyRx (V 8.0) and energy minimization was prepared using Open Babel (Version 2.3.1)[21] module of the same software. The binding energies between receptor and the ligands are attained in terms of Kcal/mol. Energy minimization was done via the Universal force field (UFF) using the conjugate gradient algorithm. A total number of 200 steps were set and the number of steps to update was set to 1. The minimization was set to stop at an energy difference of less than 0.1 Kcal/mol.[22]

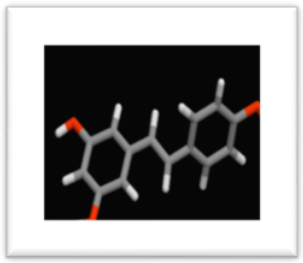
Drug screening using PyRx (V 8.0)

All the 42 phytochemicals are subjected to Drug Screening process against 1QCF receptor using PyRx (V 8.0) standalone virtual screening software.

Table 1: Molecular Properties & Drug likeness of Selected Ligands.

Sr. No	PubChem ID	Name	MW g/mol	H-Bond Donors	H-Acceptors	CLogP	Drug likeness
1	445154	Resveratrol	228.24	3	3	3.1	-1.6732
2	164676	Tanshinone 2A	294.3	0	3	4.3	-7.7862
3	5281793	Salvianolic acid	494.4	7	10	3.9	-3.8118
4	5281605	Baicalein	270.24	3	5	1.7	0.28194
5	253193	Pheophorbide	592.7	3	9	2.7	-3.1751
6	2353	Berberine	336.4	0	4	3.6	-2.2467
7	10077207	Oroxin B	594.5	9	15	-1.3	-3.2099
8	139068057	Xanthone V1	426.5	4	7		-2.1606
9	10639	Physcion	284.26	2	5	3	-0.97635
10	5378723	Vismiaquinone	352.4	2	5	4.8	0.00667
11	10331844	Napabucasin	240.21	0	4	2.3	0.56563
12	5785070	Geranyloxyemodin	406.5	2	5	6.4	-2.7783
13	10032468	Actein	676.8	4	11	3.3	-4.8009
14	9794159	S- allylmercaptocysteine	193.3	2	5	-2	-9.4523
15	5318517	Andrographolide	350.4	3	5	2.2	-4.5926
16	13342	vinblastine	811	3	12	3.7	4.4843
17	249332	Vincristine sulfate	923	5	16		5.1845
18	119034	Asiatic acid	488.7	4	5	5.7	-5.9983
19	40305	Fagaronine	350.4	1	4	4.4	-2.1697
20	638278	Isoliquiritigenin	256.25	3	4	3.2	0.13358
21	10177	Indirubin	262.26	2	3	2.7	1.8559
22	442009	Carnosol	330.4	2	4	4.4	-3.8329
23	65064	Epigallocatechin-3-gallate	458.4	8	11	1.2	-0.32874
24	5280961	Genistein	270.24	3	5	2.7	-0.09385
25	5280343	Quercetin	302.23	5	7	1.5	-0.08283
26	5944	Cantharidin	196.2	0	4	0.6	-15.812
27	99474	Diosgenin	414.6	1	3	5.7	0.84396
28	6918774	Corosolic acid	472.7	3	4	6.4	-3.8931
29	122784	Brucein D	410.4	5	9	-2.4	-3.0627
30	91466	Matrine	248.36	0	2	1.6	0.2541
31	122724	Celastrol	450.6	2	4	5.9	-1.5186
32	10328746	Ardisiacrispins A	1061.2	12	22	-1.1	-15.93
33	73412	Madecassic acid	504.7	5	6	4.4	-5.9983
34	10281	Thymoquinone	164.2	0	2	2	-1.1996

35	3086007	Ginsenosides	444.7	2	2	8.5	-1.3
36	10205	Plumbagin	188.18	1	3	2.3	-1.4992
37	73611	Solamargine	868.1	9	16	1.1	3.1642
38	6474554	Rhinacanthin C	410.5	1	5	5.3	-9.4518
39	11968867	Asperulosidic acid	432.4	6	12	-3.2	-2.335
40	265237	Withaferin A	470.6	2	6	3.8	1.6889
41	4501	Nitidine	348.4	0	4	4.6	-2.1842
42	92023653	Fucoidan	242.25	3	7	-1.5	-0.04317
43	10235	Cytisine	190.24	1	2	0.2	3.1571
44	129320386	Armillarikin	464.9	3	7	4.1	-1.3436
45	5281852	Bilobol	318.5	2	2	8.1	-21.029
46	76617	Adipostatin A	320.5	2	2	9	-20.191
47	445354	Retinol	286.5	1	1	5.7	-3.4867
48	251690	O- orsellinaldehyde	152.15	2	3	1.6	-4.177
49	5281727	Pterostilbene	256.3	1	3	3.8	-1.4702
50	393472	Acetogenins	470.6	4	7	3.9	-17.287
51	70698023	Longikaurin A	348.4	3	5	1.4	-7.3568



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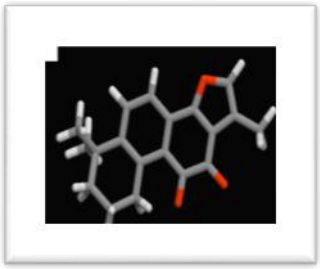
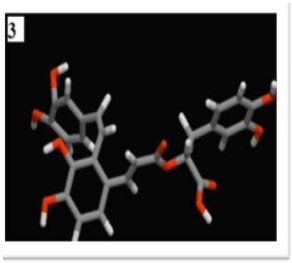
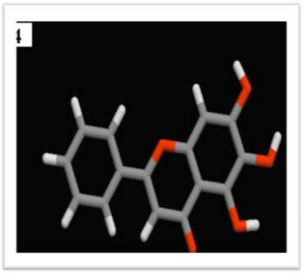


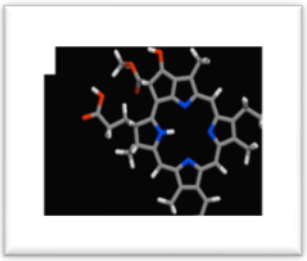
Tanshinone 2A



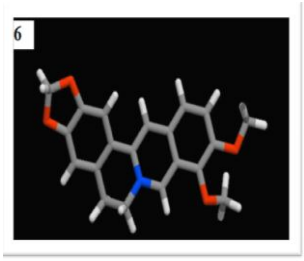
Salvianolic acid



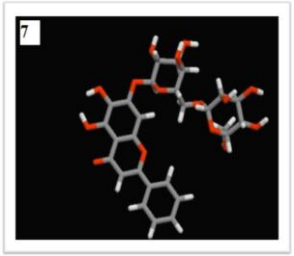
Baicalein



Pheophorbide



Berberine



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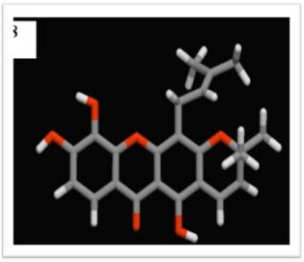
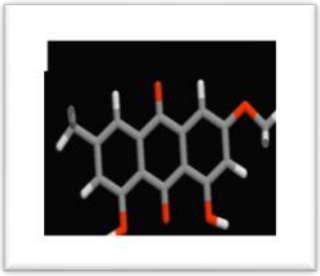


Xanthone V1



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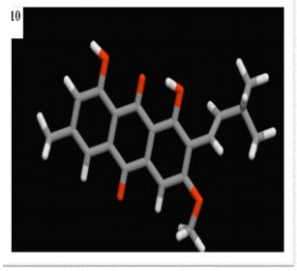

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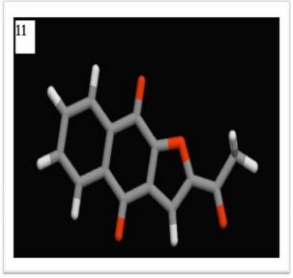

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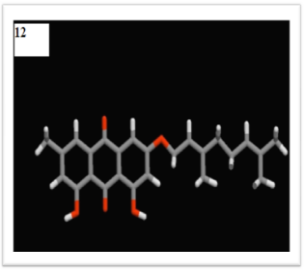

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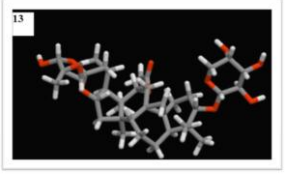

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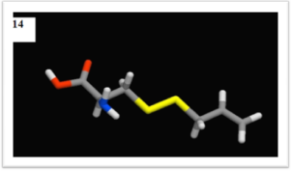

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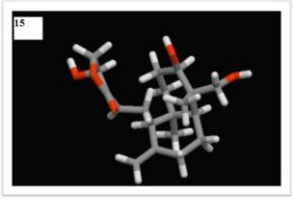
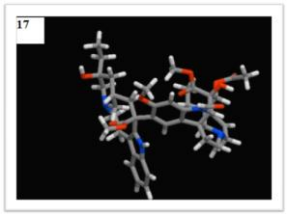


Andrographolide

vinblastine



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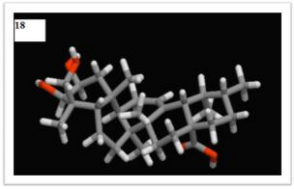

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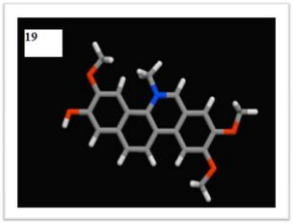

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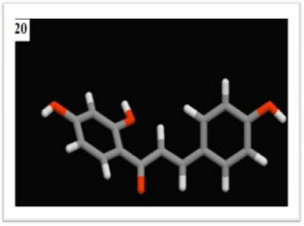

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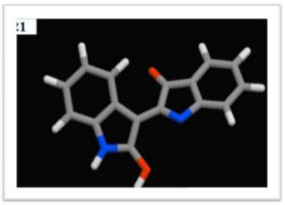

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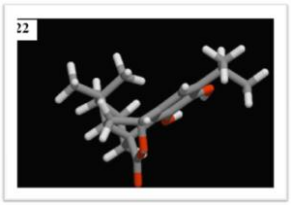

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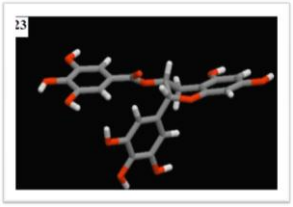

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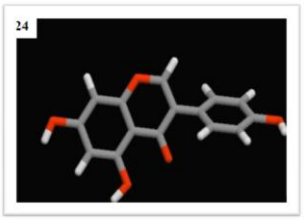

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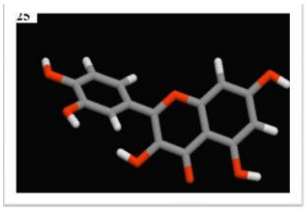

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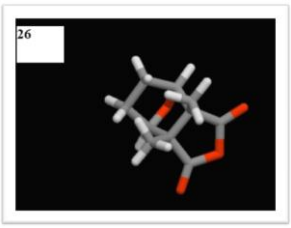

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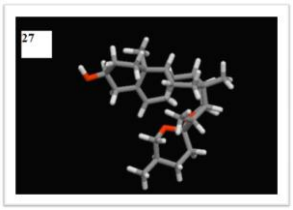

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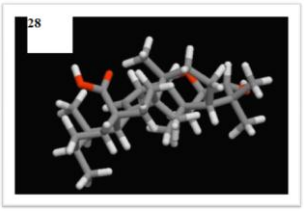

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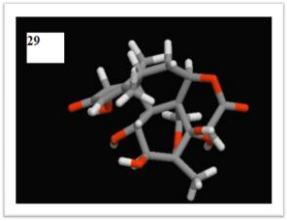

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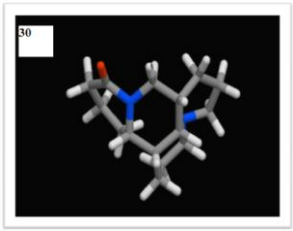

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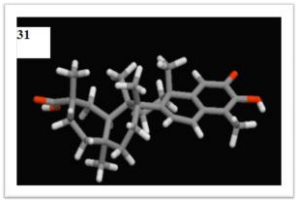
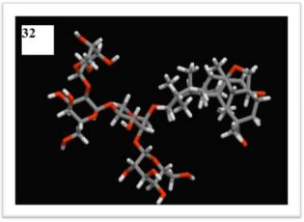


Celastrol



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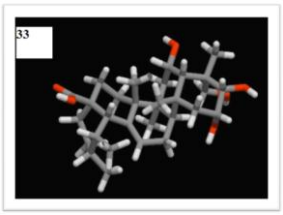
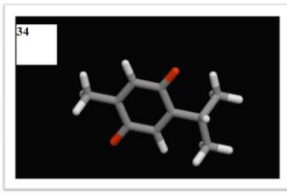


Madecassic acid



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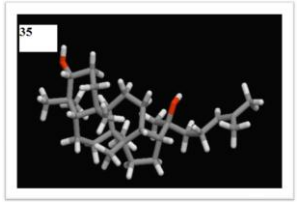

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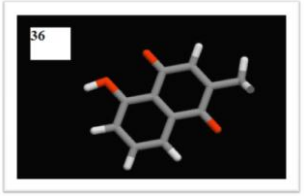
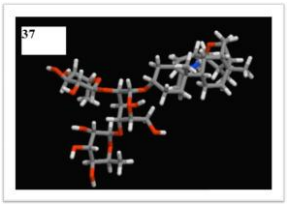


Plumbagin



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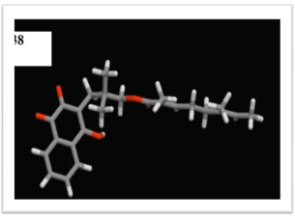

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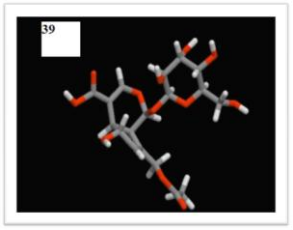

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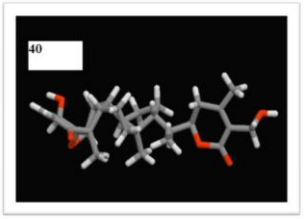
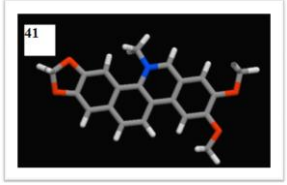
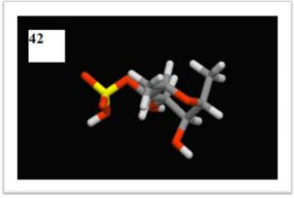


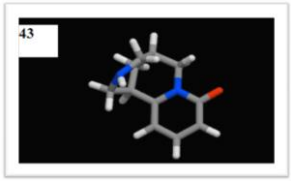
Withaferin A



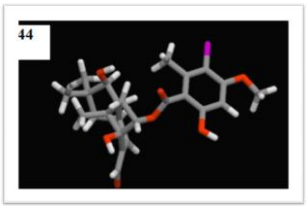
Nitidine



Fucoidan



Cytisine



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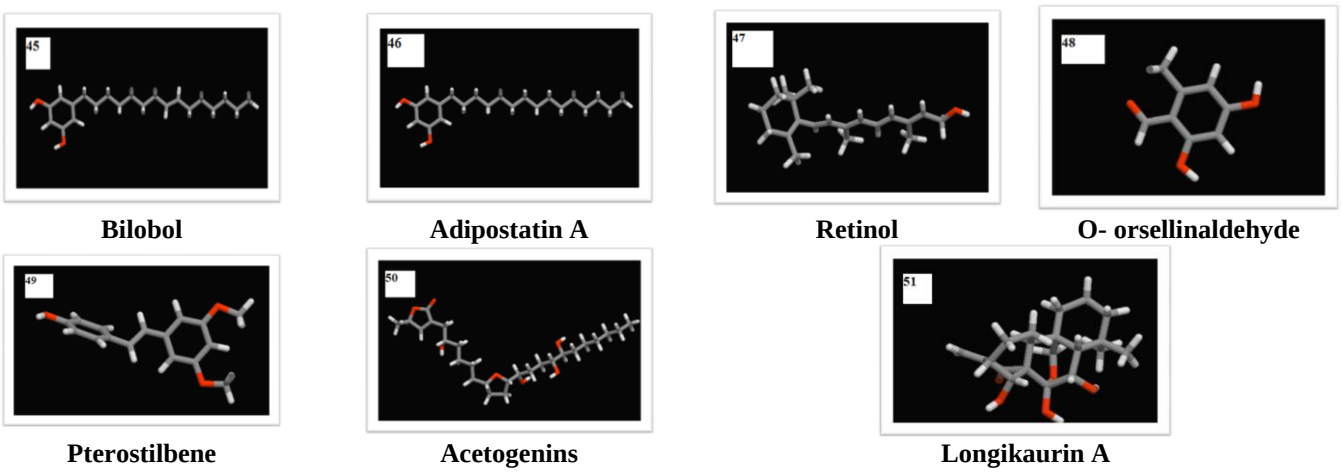


Figure 2: 3D structures of the 51 ligands used in the experiment.

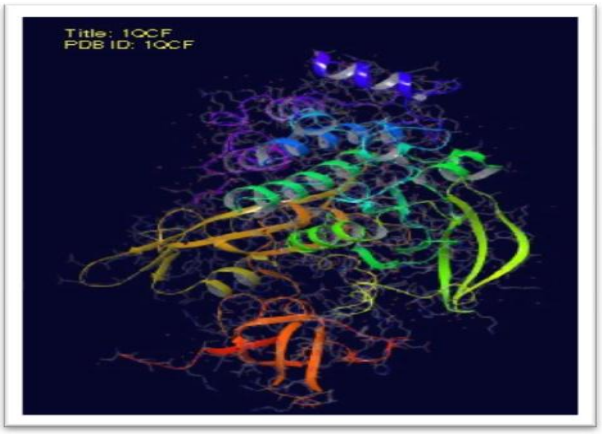


Figure 3: The 3D structures of the target proteins, Tyrosine Kinase. The 3D structures were visualized by Discovery Studio Visualizer.

Molecular Docking studies

Molecular docking is a method used to analyze the position and the inhibition interaction between the protein and the small molecules. Molecular docking was executed with PyRx (V 8.0), which is an extension of the python molecular viewer. A Lamarckian genetic algorithm was used to perform the automated molecular docking of the protein with each ligand. The torsion bonds and side chains were kept to rotate freely, while the protein structure was kept rigid. As a reprocessing step, the PDB format of macromolecule and SDF format of small molecules are converted to PDBQT format. Gasteiger charges were computed, and all the charges of non-polar hydrogens were assigned.[23] The docking of small molecules to the macromolecule was focused on the specific binding site. The total number of rotatable bonds of the ligand is calculated. The grid was defined to the binding site of the protein structure with the configurations of x/y/z coordinates was set to size x= 59.60, size y=76.61 and z=69.28 centre of the grid box was set to centre x= 12.53, centre y=29.84, centre z=32.10 in X, Y, Z dimensions, in which the grid was covered to the binding site of macromolecule and the grid was spaced at 0.375 Å.

ADME and Toxicity predictions

The selected 51 phytochemical compounds were further studied for their Adsorption, distribution, excretion, metabolism, and toxicity profile using SWISS ADME[24] and data warrior tools.[25,26] The predicted properties considered were blood-brain barrier penetration properties, Human intestinal absorption, inhibition to cytochrome P450 enzyme, and bioavailability. Compounds showing satisfactory properties were further studied for their toxicity profile using data warrior tools. Toxicity profiles included were mutagenicity, tumorigenicity, irritability, reproducibility, Ames toxicity, and carcinogens.

Molecular dynamic simulation

Docked protein and ligand complexes were subjected to molecular dynamics simulation using NAMD software.[27] The success of MD simulation depends on the selection of the initial protein and ligand structures. Initially, the structure was checked for inconsistencies. Out of 51 selected compounds from the docking results, we have selected the three final compounds having a PubChem number 9794159, 10235 and 393472. The docked complexes were studied for their stability during the simulation. The root means square deviation, root mean square fluctuation, and radius of gyration was studied for protein backbone residue and ligand within the binding site of the simulated system.[28-30] The stabilities of the complexes were examined by monitoring their root mean square deviation (RMSD) during 50, 00,000 steps for a 10 ns simulation. MD simulations were performed using the CHARMM36 force field.[31] Visual molecular dynamics (VMD) was used to generate PSF files for complex. All complex was solvated in cubic water boxes containing transferable intermolecular potential with 3 points (TIP3P) water molecules. The box size was chosen to match the molecular dimensions so that there was a distance of 5˚A between the protein surface and the edges of the periodic box. A 5˚A cut off distance was used to calculate short-range non bonded interactions. The particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions. The SHAKE method was used

to constrain all bonds involving hydrogen atoms. A conjugated gradient system was used for energy minimization, with all parameters set to default. The system first performed 10000 steps of conjugated gradient with energy minimization. We used Langevin dynamics with pressure control so our system was not an NVT ensemble. The Nose–Hoover method was used to maintain a constant temperature. The time step of each simulation was set to 2 fs.[27, 32, 33] Visualizations and data analysis were performed with VMD software.[34]

3. RESULTS AND DISCUSSION

Virtual screening and docking results

Virtual screening helps us to screen the biological molecules with good binding affinity. In this study, we have used PyRx 8.0 tool to screen out the molecules. A total of 51 natural ligands were selected and were docked to the target protein. The docked compounds were examined in the Auto dock tool and binding free energy was calculated.[35] (Table 2).

Table 2: ADMET analysis with the Lowest Binding affinity.

PubChem ID	BBB Penetration	HIA	CYP2D6 Inhibitor	BA	Mutagenic	Tumorigenic	Reprodu-ctive effect	Irritant
445154	Yes	High	No	0.55	none	none	high	none
164676	Yes	High	Yes	0.55	none	none	high	none
5281793	No	Low	No	0.11	none	none	high	none
5281605	No	High	Yes	0.55	none	none	none	none
253193	No	Low	No	0.56	none	none	none	none
2353	Yes	High	Yes	0.55	none	none	none	none
10077207	No	Low	No	0.17	none	none	none	none
139068057	No	High	No	0.55	high	none	high	none
10639	No	High	No	0.55	low	none	none	high
5378723	No	High	No	0.55	low	none	none	high
10331844	Yes	High	No	0.55	none	none	none	none
5785070	No	High	No	0.55	low	none	none	high
10032468	No	Low	No	0.17	none	none	none	none
9794159	No	High	No	0.55	none	none	none	none
5318517	No	High	No	0.55	none	none	none	none
13342	No	Low	No	0.17	none	none	none	none
249332	No	Low	No	0.17	none	none	none	none
119034	No	High	No	0.56	none	none	none	none
40305	Yes	High	Yes	0.55	none	none	none	none
638278	Yes	High	No	0.55	high	none	none	low
10177	Yes	High	Yes	0.55	none	none	none	none
442009	Yes	High	No	0.55	none	none	none	none
65064	No	Low	No	0.17	none	none	none	none
5280961	No	High	Yes	0.55	high	high	high	none
5280343	No	High	Yes	0.55	high	high	none	none
5944	Yes	High	No	0.55	none	high	none	high
99474	Yes	High	No	0.55	none	none	low	none
6918774	No	High	No	0.56	none	none	none	none
122784	No	Low	No	0.55	none	none	low	none
91466	Yes	High	No	0.55	none	none	none	none
122724	No	Low	No	0.85	none	none	none	none
10328746	No	Low	No	0.17	none	none	none	high
73412	No	High	No	0.56	none	none	none	none
10281	Yes	High	No	0.55	high	none	none	none
3086007	No	Low	No	0.55	none	none	none	high
10205	Yes	High	No	0.55	high	none	high	none
73611	No	Low	No	0.17	none	none	low	none
6474554	No	High	Yes	0.56	none	none	high	high
11968867	No	Low	No	0.11	none	none	none	high
265237	No	High	No	0.55	none	none	low	none
4501	Yes	High	No	0.55	none	none	none	none
92023653	No	High	No	0.56	none	none	none	none
10235	No	High	No	0.55	none	none	none	none
129320386	No	High	No	0.55	none	high	none	high

5281852	No	High	Yes	0.55	none	none	none	none
76617	No	High	Yes	0.55	none	none	none	none
445354	Yes	High	No	0.55	none	none	none	none
251690	Yes	High	No	0.55	none	none	none	none
5281727	Yes	High	Yes	0.55	none	none	high	none
393472	No	High	No	0.55	none	none	none	none
70698023	No	High	No	0.55	none	none	none	high
445154	Yes	High	No	0.55	none	none	high	none

ADMET analysis

We have selected 51 compounds and the same compounds were studied for their ADMET properties. The properties like Human intestinal absorption, irritability, reproductive effect, inhibition to cytochrome P_{450} enzyme, and several others were predicted. It was clear from the results that from the selected compounds with high intestinal absorption values and negative inhibitory actions to cytochrome P450 enzymes, compounds were also studied for their mutagenic, tumorigenic, irritability, and reproductive effect. Table no 2 indicates that compounds number 5350, 969516 have either of the said effects, so these compounds were also removed from the study. Finally, we selected one ligand namely Cytisine CID 10235 for further analysis. The ligand selected for further study was having either hydrogen bonds or presents a hydrophobic interaction with the protein Table 3 and 4.

Table 3: Hydronic Interaction between Protein and Ligand Complex.

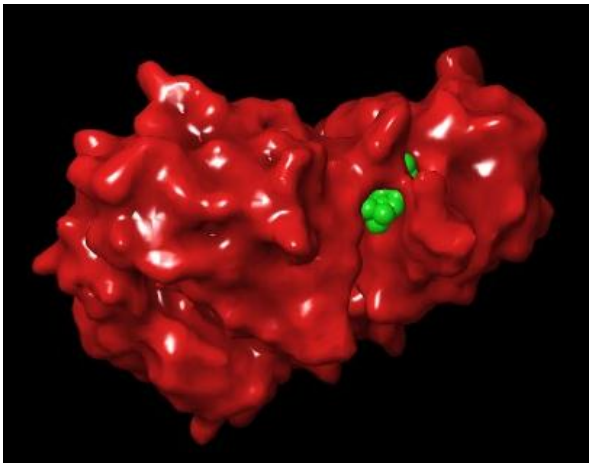
RESNR	RESTYPE	RESCHAIN	RESNR_LIG	RESTYPE_LIG	RESCHAIN_LIG	DIST	LIGCARBONIDX	PROTCARBONIDX	LIGCOO	PROTCOO
273	LEU	A	1	UNK	N	3.75	4451	1924	26.815,28.766,37.334	29.766,27.006,38.846
281	VAL	A	1	UNK	N	3.93	4444	1985	28.666,32.312,39.875	28.418,29.973,43.019
293	ALA	A	1	UNK	N	3.71	4452	2113	25.711,28.753,38.183	25.594,25.503,39.961
340	PHE	A	1	UNK	N	3.62	4451	2554	26.815,28.766,37.334	27.615,25.536,35.906

Table 4: Hydrogen bond interaction between Ligand and Protein complex.

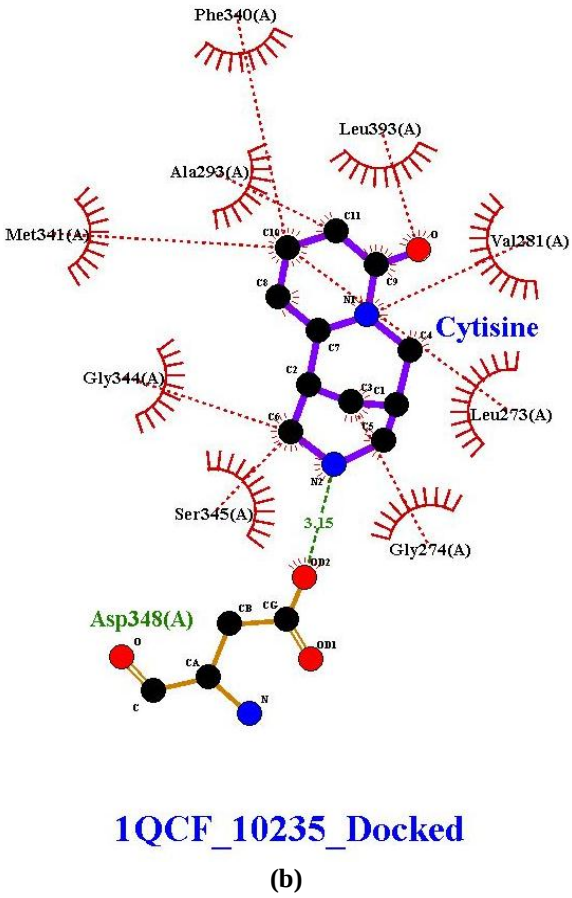
Resnr	Restype	Reschain	Resnr_LIG	Restype_Lig	Reschain_Lig	Sidechain	Dist_H-A	Dist_D-A	Don_Angle	Protisdon	Donoridx	Donortype	Acceptoridx	Acceptortype	Ligcoo	PROTCOO
177	SER	A	527	PTR	A	True	2.31	3.19	154.50	True	962	O3	4400	O3	-1.647,30.848,17.757	-3.568,28.592,18.942
178	GLU	A	527	PTR	A	False	1.84	2.82	161.20	True	965	Nam	4388	O3	-0.403,29.677,19.591	-1.823,28.739,21.841
179	THR	A	527	PTR	A	True	1.77	2.70	163.96	True	980	O3	4386	O3	-1.123,32.061,19.911	-3.354,33.523,20.354
179	THR	A	527	PTR	A	False	3.06	4.02	157.88	True	975	Nam	4388	O3	-0.403,29.677,19.591	-3.534,30.960,21.759
185	SER	A	527	PTR	A	True	2.04	2.89	147.58	True	1038	O3	4400	O3	-1.647,30.848,17.757	-1.223,28.296,16.473
185	SER	A	527	PTR	A	True	2.85	3.51	126.17	False	4388	O3	1038	O3	-0.403,29.677,19.591	-1.223,28.296,16.473
529	GLU	A	527	PTR	A	True	3.37	3.96	123.70	True	4420	O3	4392	O2	-1.501,36.358,11.180	-5.141,35.261,10.062

Protein-ligand interaction

The hydrogen bond and hydrophobic interactions of protein-ligand complexes were analyzed by LigPlot+ (v 1.4.5)38 and Protein-ligand interaction profiler. "LigPlot+" is a graphical system that generates multiple two-dimensional (2D) diagrams of ligand-protein interactions from docked complexes. PLIP is complementary to another state of the art tools like a SWISS dock, galaxy site, or ProBis and thus it can be used to study the protein-ligand complex. The server allows comprehensive detection and visualization of protein and ligand complexes along with interaction patterns.



(a)



(b)

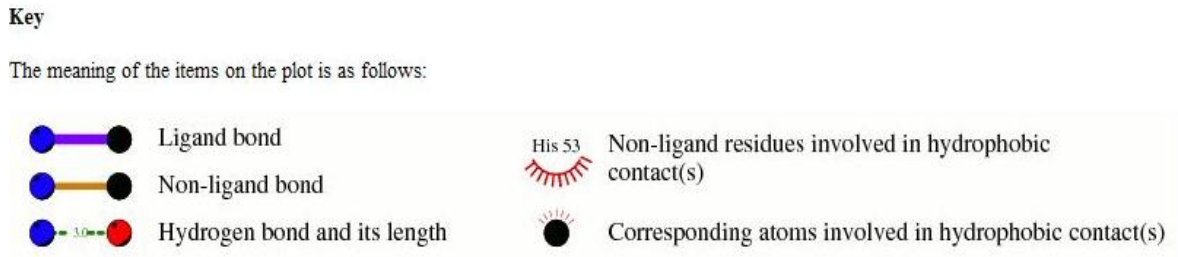


Figure 4 (a) and (b):2D representations of the best pose interactions between the best ligands and protein.

Molecular Dynamic Simulation studies

We assessed the residue RMSD to study the residue behaviour of the protein during the simulations. In general, a residue's RMSD value was considered to represent the local flexibility of a protein and ligand complex. It reflected the mobility of an atom during the MD simulation trajectory. Therefore, a higher residue RMSD value indicated higher mobility; conversely, a lower residue RMSD value indicates lower mobility. To investigate the fluctuations in the ligand-binding energy as well as the motions of the amino acid residues within the complex during the simulation, the root means square fluctuation (RMSF) of the complex was also monitored. Besides, the compactness of each complex was determined by carefully examining how folded or unfolded the protein-ligand complex was by calculating the radius of gyration.[33] Based on the docking analysis 51 compounds were selected for further ADMET investigation and it leads us to select the final compound Cytisine to consider the structural stability of each protein-ligand complex by molecular dynamic simulation. The stability of complex (1QCF-Cytisine) was monitored using root mean square deviation (RMSD) during 10 ns simulation studies.

Table 5: RMSD values for the simulated complexes.

Protein-Ligand complex	Mean RMSD (Å)	Min RMSD (Å)	Max RMSD (Å)
1QCF-Cytisine	1.9627	0.4300	3.7910

The values presented in (Table 5) for protein-ligand complex studied for its stabilities during 10 ns simulation. From the values, it is clear that the range of RMSD obtained for the complex follows the acceptance

range between 1 to 3.5 (Å). It is also observed from the graphs that the complex was also equilibrated as the average RMSD values are stabilized at the end of the 10 ns simulation. This fixed range of RMSD was indicating the interaction between bound ligand and flexible loop region, as it reduces the flexibility of the protein-ligand complex.

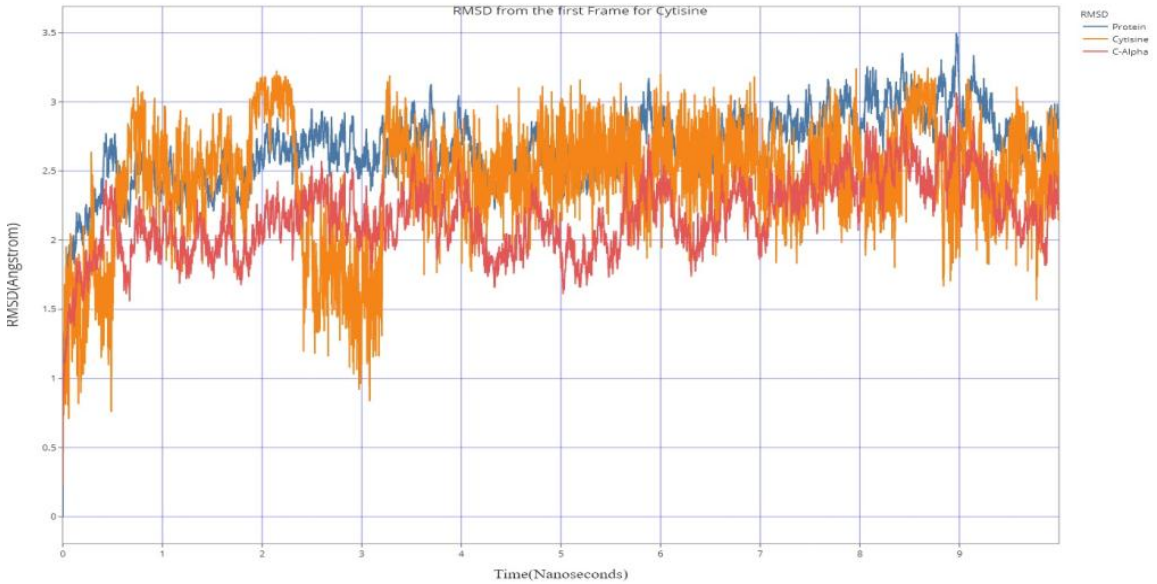


Figure 5: RMSD results for Cytisine with 1QCF protein, based on 10 ns simulation.

The root means square fluctuations (RMSF) were assessed and plotted to equate the flexibility of each residue in the–ligand-protein complexes. The RMSF of the protein-ligand complex denoted the minimized fluctuation for all the complexes. The RMSF did not deviate much during the simulation period of 10 ns and the average RMSF values were kept constant for all the complexes. The radius of gyration was also monitored during the 10-ns MD simulation for each protein-ligand complex to ascertain whether the complex was stably folded or unfolded. If the radius of gyration remained relatively constant, the complex was considered to be stably folded, otherwise, it was considered to be unfolded.

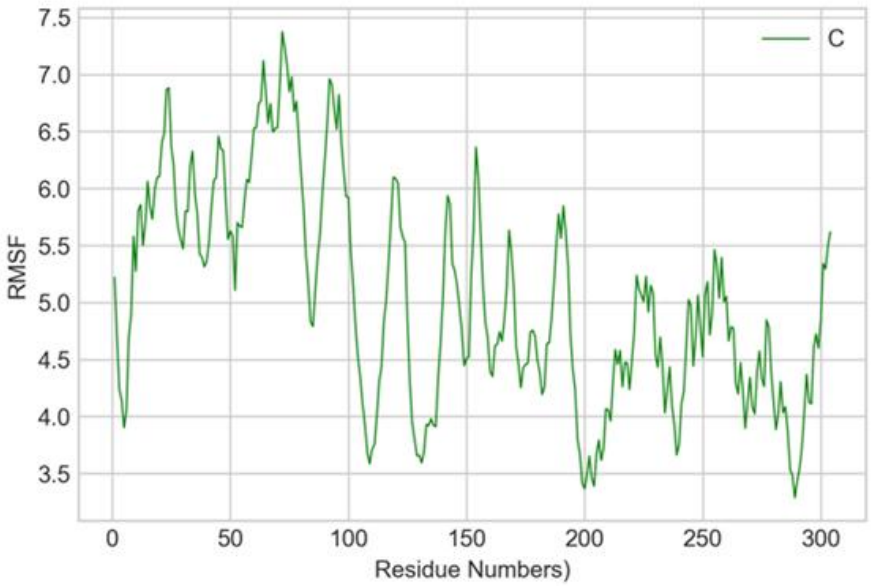


Figure 6: RMSF results for Cytisine acid with 1QCF protein, based on the data from 10 ns simulation.

Table 6: RMSF values for the Protein- Ligand complex.

Protein-Ligand Complex	Mean	Min	Max
1 QCF-Cytisine	4.8507	2.5665	7.4256

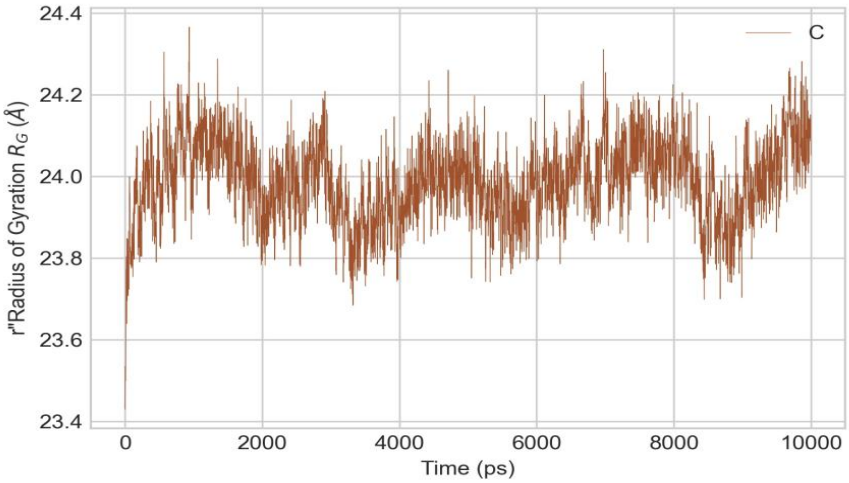


Figure 7: The radius of Gyration results for Cytisine with 1QCF protein, based on the data from the 10 ns simulation.

In this study, the radius of gyration value obtained is listed in Table 6. The values obtained for the Cytisine showed a relatively constant radius gyration during the simulation. So, we can conclude that the complex is formed relatively stable folded polypeptide structures during the 10 ns MD simulation

4. CONCLUSION

The globe is moving on the way to use natural products due to their low cost and trustworthiness over side effects resulted from existing drugs. Researchers are raising their efforts for the development of phytopharmaceuticals against severe metabolic syndromes including cancer. Bioactive phytochemicals/formulations are potential leads for the development of safer anticancer drugs. Several plants and their constitutive phytochemicals have been screened for this purpose but only a very few have reached up to the clinical level. Anticancer phytochemicals described in this article must be further researched in clinical trials for their effectiveness and toxicological documentation. They must be developed as druggable forms with sufficient bioavailability. Moreover, we know that a traditional herbal preparation has greater medicinal effect than the same phytochemical/molecule taken in a pure form. So therapeutic intervention based upon the combination of anticancer molecules may give potent and effective therapeutic results. The treatment of chronic disease like cancer with phytochemicals is critical in the research studies. Our in silicon approach on phytochemicals against cancer target 1QCF is carried out using virtual screening, molecular docking and ADMET methods. Virtual screening of three compounds showed the binding affinity towards target 1QCF. The compounds were screened with least binding affinity and compound Cytisine was selected as hits. The molecular docking of the hit showed the binding mode and interaction energy. H- bond pattern was analyzed and confirmed the inhibition of cancer target 1QCF to show the anti cancer activity of phytochemicals. Our result based on in silico studies, concluded that Cytisine inhibit 1QCF and possessed better anticancer activity against 1QCF. Further studies on lead Cytisine can be done in experimental studies to confirm the inhibition and may be used in the treatment of cancer.

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Conflict of Interest: None of the authors have any conflicts of interest.

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