MOLECULAR DOCKING OF SELECTIVE PHYTOCHEMICALS FOR ACETYLCHOLINE ESTERASE INHIBITION

Submitted in fulfillment of the requirements for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

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MAY 2021

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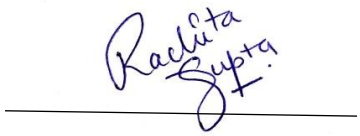
DECLARATION BY STUDENTS

We hereby declare that the project work entitled "Molecular Docking of selective phytochemicals for acetylcholine esterase inhibition" submitted to the Department of Biotechnology and bioinformatics ,Jaypee University Of Information Technology Solan(H.P), is a bonafide record of original work done by us . The work was carried out under the supervision of Dr.Udaybanu Malairaman.

Manvhi Rastogi(171834)

Rachita Gupta(171818)



Department of Biotechnology and Bioinformatics
Jaypee University Of Information Technology
Waknaghat (H.P)

Date: May 25, 2021

This is to certify that the above statement made by the student is true to the best of my knowledge.

SUPERVISOR'S CERTIFICATE

This is to certify that the work titled " Molecular Docking of selective phytochemicals for acetylcholine esterase inhibition "by Manvhi Rastogi and Rachita Gupta during the end semester in June 2021 in fulfilment for the award of degree of Bachelor of Technology in Biotechnology of Jaypee University of Information Technology ,Solan has been carried out under my supervision .This work can be sent totally or partially to any other university or college to obtain any degree or recognition.

Signature of Supervisor

Name of supervisor: Dr Udaybanu Malairaman
Designation: Associate Professor

Department of Biotechnology and Bioinformatics
Jaypee University of Information Technology
Waknaghat, Solan(H.P.)

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We sincerely thank the entire Department of Biotechnology and Bioinformatics for granting us a friendly license in the research work and for providing us with the necessary administrative facilities.

Date : May 25, 2021

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ABSTRACT

Neurodegenerative is derived from two words- "neuro- referring to neurons" and "degenerative- meaning dying". It collectively refers to death of neurons or nerve cells, thereby resulting in impaired communication between the brain cells. Many neurodegenerative diseases have found their way in human lives like, Alzheimer's, Parkinson, Huntington's disease and others.

Most common of these happen to be Alzheimer's disease (A.D.), a form of dementia, which progresses gradually with time and age, affecting memory, thinking and behaviour of the affected individual. It is characterized by deficiency of Acetylcholine and deposition of Beta-amyloid as plaques.[1] Globally there happens to be no way of completely curing the disease, instead the drugs approved tend to slow down the progression of the disease, and cure some of the symptoms.

In this study we aim to screen out some potential phytochemical molecules , by using computational tools (like molecular docking), which might tend to inhibit the activity of acetylcholinesterase, which hydrolyses the acetylcholine molecule into acetate and choline, ultimately leading to A.D.[2] Using Rivastigmine as the standard molecule, we will try to compare several classes of phytochemicals having the potential to act as Acetylcholinesterase inhibitor (AChEi).

Keywords: Neurodegenerative disorders, Alzheimer's Disease, phytochemicals, molecular docking, acetylcholinesterase, inhibitors

CHAPTER 1: NEURODEGENERATIVE DISEASES

Neuro-degenerative illnesses are a diversified group of diseases which can be described by the gradual degradation of the structure and capacity of the central sensory system or peripheral sensory system. These are caused because of the loss of function of nerve cells present in peripheral nervous system and brain leading to their death. It is observed that many of the degenerative conditions are affected by environmental factors while some may arise due to genetic susceptibility. The classical neuro-degenerative ailments, Alzheimer's disease and Parkinson's disease are related to the old population. We know that life expectancy has been increasing with time and so does the problem and probability of neurodegenerative diseases. Currently, there is no way to recede progression of disease and known cures. However treatments may help in soothing some of the bodily or psychological symptoms linked with neurodegenerative ailments. [6]

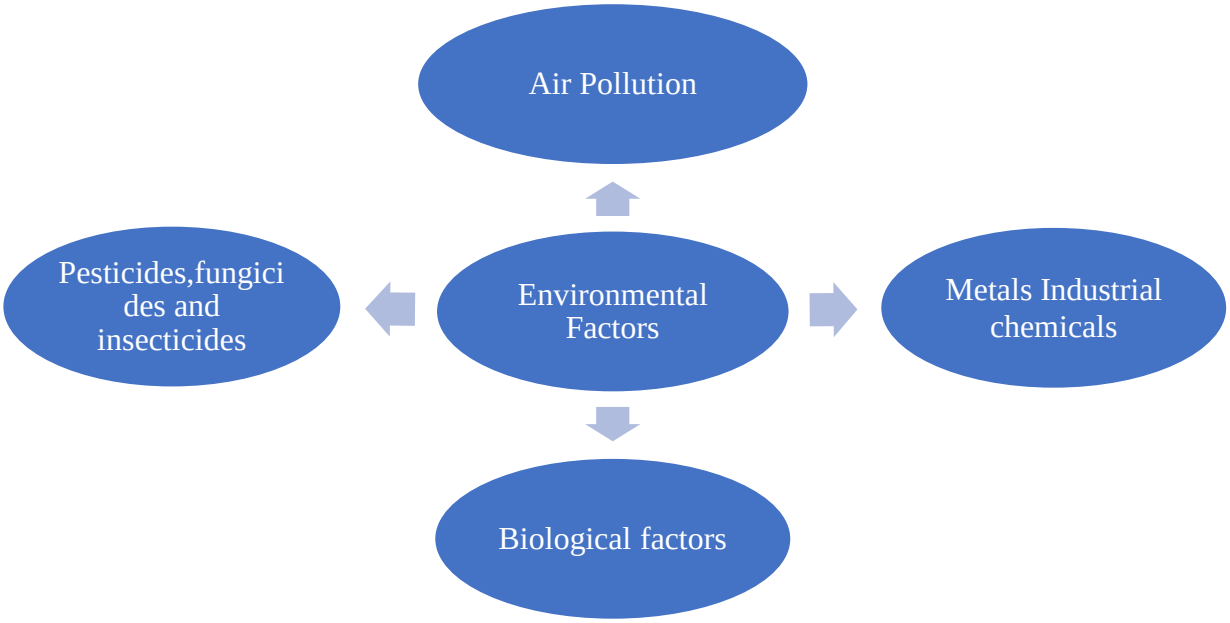


Fig.1: Environmental factors known to influence neurodegenerative diseases.

1.1 ALZHEIMER'S DISEASE

Alzheimer's disease is a gradual degenerative disease which can cause cells of the brain to degenerate (waste away) and expire. This disorder is the conventional cause of dementia with continuous decrease in thinking, conduct, communication, interpersonal and soft skills can

undermine an individuals' ability to function independently. It occurs when there is a formation of plaques in the brain which contain beta-amyloid cells.[10] In this ailment as symptoms become severe, it is tough for an individual to recognize recent events, reason about that and to remember people they know.

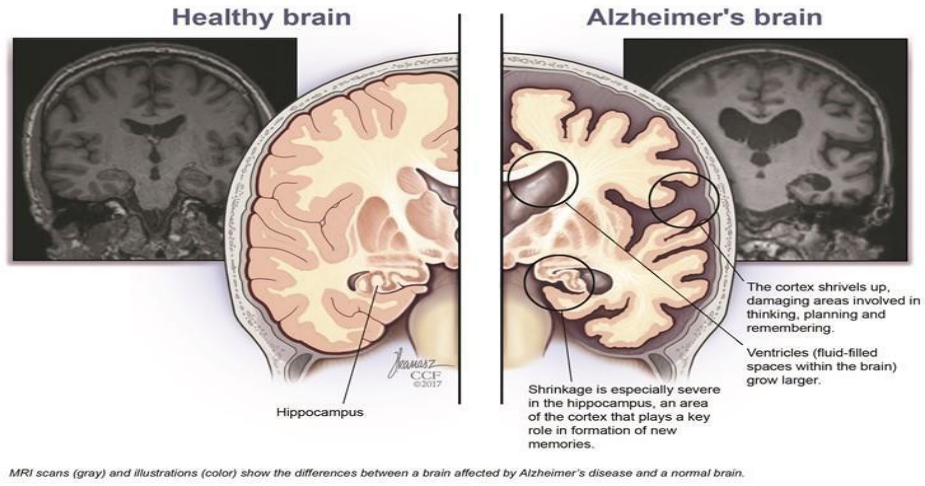


Fig 2: Healthy brain vs AD brain.

The most suitable treatment for AD and other types of dementia is to recover acetylcholine levels by inhibiting two main forms of cholinesterase: Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE). AChE prevails (80%), while BuChE is plays a minimal role in modulating ACh concentration in the brain.[7] BuChE is a non-specific cholinesterase enzyme that can hydrolyze many different choline esters. It is alike to the neuronal acetylcholinesterase.[14]

BuChE balances acetylcholine levels inside the hippocampus and temporal cortex. AChE is a cholinergic enzyme, mainly found in the postsynaptic neuromuscular junction, but in muscles and nerves as well.[10] The natural neurotransmitter acetylcholine (ACh) is hydrolyzed into acetic acid and choline.[9] There is a cholinergic hypothesis according to which, the inhibition of AChE can catalyze the hydrolysis of acetylcholine and expand the quantity of acetylcholine in the brain, thus enhancing the cholinergic function of AD patients. [11]

Although synthetic drugs, like donepezil, neostigmine, and rivastigmine can be used to treat AD symptomatically. The search for novel compounds from natural products such as phytochemicals has attracted a lot of attention. [1] Although these drugs cannot completely cure

the disease. In addition, these drugs also have concomitants such as nausea, vomiting, headache, stomach cramps, and insomnia.

Therefore, many plant components used in various traditional medicine systems have been tested as memory enhancers to determine anticholinesterase (AChEi) activity. [6][14] Most of these AChEi are alkaloids, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. On the other hand, terpenoids, flavonoids and other phenolic compounds are several effective non-alkaloidals and potent AChEi are obtained from natural sources. [4]

1.2 PARKINSON'S DISEASE

Parkinsons' is a neuro-degenerative disorder specified by stiffness, trembling and difficulty in movements and coordination. It usually arises due to the impairment of the nerve cells or the neurons, responsible for controlling movement. Under normal circumstances these nerve cells produce a chemical known as dopamine, which is responsible for transmitting nerve signals, across the nerve impulses. When the nerve cells/neurons get impaired, they produce low amounts of dopamine, which leads to coordination difficulties and lead to impaired movements.

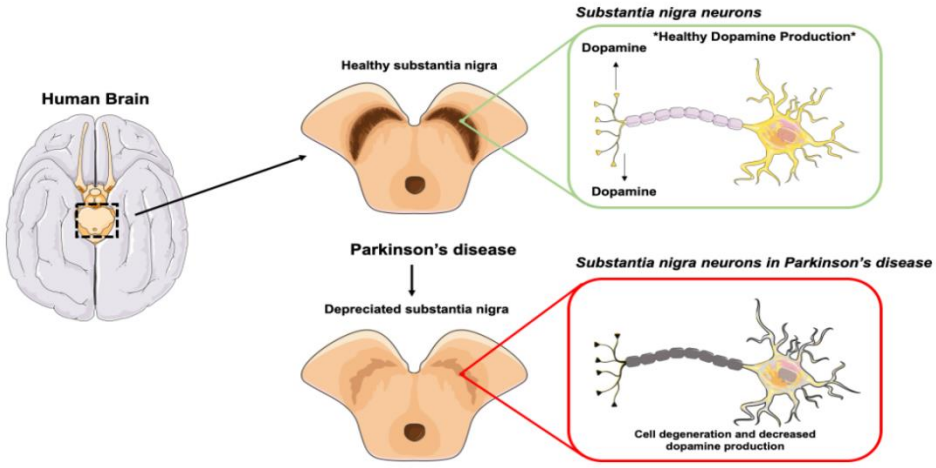


Fig 3: Human Brain depicting Parkinson Disease

Parkinson's disease and Alzheimer's disease have some overlapping symptoms. Memory and language are affected in Alzheimer's disease, and Parkinson's disease generally affects an individual's analytical ability. Parkinson's disease is marked by the presence of Lewy bodies,

which are sticky bumps of proteins present in the brain, while Alzheimer's disease is distinguished by the appearance of β-amyloid plaques in the brain.

1.3 OBJECTIVE

In this project we will be mainly focusing on inhibition of acetylcholinesterase by docking of various phytochemicals on AChE;

1) Review some of the well-established drugs and phytochemicals that help in treating AD.

2) By evaluating the rationality of the interaction between the target and acetylcholinesterase, filtering and verifying the potential targets identified by the target-centric molecular docking method.

3) Based on the predicted interactions of acetylcholinesterase, through tests on all the structures contained in various databases, new potential phytochemicals that can be used as anticholinesterases are to be discovered.

CHAPTER 2: LITERATURE REVIEW- PHYTOCHEMICALS

Plants not only produce primary metabolites, apart from it they also produce secondary metabolites like Phytochemicals, which are non-nutritive chemical compounds produced by plants using various chemical pathways.[16][17][19] These compounds generally help to tackle fungal, bacterial and plant viral infections, apart from this they also exhibit various medicinal properties which can be used as a potential treatment for certain diseases.[20] They usually belong to alkaloids, flavonoids, terpenoids etc. classes.[21][22][23][24]

We have tried to investigate various phytochemicals (like, Quercetin, Resveratrol, Huperzine A, Galantamine, Bacosides, Assoanine, N-methylasimilobine, Fumuranine, Berberine) and their roles in improving the severe conditions of Alzheimer's.

Rivastigmine, a dual AChE and BuChE inhibitor is a semi-synthetic drug (a derivative of physostigmine) used to slow the progression of dementia and other related issues with Alzheimer's disease. Rivastigmine was observed to exhibit inhibitory effects against AChE in both pre-clinical and clinical studies, when delivered as transdermal patches. These dual inhibitory activities of rivastigmine make it a potential therapeutic agent against neurodegenerative ailments.[7][10]

Berberine is a naturally occurring isoquinoline alkaloid compound obtained from *Rhizoma coptidis* (Chinese herb). It forms a major part in Chinese herbal medicines. Berberine exhibits various activities like anti-oxidant, AChE and BuChE inhibitory effect, offers a potential use in anti-Alzheimer's therapy. It can be administered orally.[5][8]

Quercetin is another plant pigment identified as a flavonoid, found in various plants like St. John's wort, Gingko biloba, apples, cherries, etc. It houses certain biological properties like antioxidant activities, anti-viral, anti-carcinogenic, anti-infection etc which tend to improve physical and mental condition of an individual. It works by protecting the neuronal cells by reducing the oxidative stress and neuroinflammation. It tends to restore the acetylcholine levels in the brain by inhibiting hydrolysis of acetylcholine by acetylcholinesterase enzyme.[1][3][25]

Assoanine happens to be a pyrollophenanthridine metabolite, isolated from *Narcissus assoanus* is an alkaloid phytochemical. It was also obtained as a degradation product of

Amaryllidaceae alkaloids. It was observed, alkaloids having galantamine skeletons tend to exhibit AChE inhibitory activities.

A derivative of assoanine: oxoassoanine belonging to phenanthridines, is a steroidal alkaloid isolated from *Narcissus assoanus* exhibiting AChE inhibition. Another derivative showing this property of inhibition: vasconine is a Amaryllidaceae alkaloid from the same plant.

Galantamine is a naturally occurring tertiary alkaloid isolated from the bulbs of *Narcissus* species. Since it is able to cross the blood brain barrier, it can be used as an anesthetic, to relieve neuropathic pains. Since galantamine modulates the presynaptic nicotinic receptors along with its proposed ability to inhibit AChE, a dual action has been claimed. It does not pose any serious side effects till date.[35]

Retusin is a type of flavonoid called o-methylated isoflavone. Fabaceae species of Dipteryx odorata, Dalbergia retusa and Millettia nitida have retusin present in them. It is very effective in inhibiting cellular melanogenesis and is known to have free radical scavenging properties. It inhibits the formation of cellular melanin thereby exhibiting the great potential in cosmeceutical products. Even though it has potential AChE inhibition properties but limted studies have been carried out on this phytochemical.

Huperzine A, a herbal extract derived from a Chinese herb *Huperzia serrata*, belongs to sesquiterpene class of alkaloids.[34] Many clinical studies performed till date claim it to be a well tolerated inhibitor of Acetylcholinesterase, which makes it a good potential molecule for treating Alzheimer’s disease. It is also found beneficial in treating memory impairment in patients sufferings from schizophrenia, vascular dementia and sleep disorders.[33]

Resveratrol is a stilbenoid, a variety of natural phenols and a phytoalexin that are processed from a variety of plants when plants are attacked by a variety of plants in response to laceration or when the plant are attacked by pathogens, such as bacteria or fungi (including Japanese knotweeds and pine trees). The possible impact of resveratrol on cognition has been evaluated. One of the research reviews concludes resveratrol to have no consequence on neurological function, but also reported that despite irregularities in study design and results, resveratrol improved cognition and mood. In a one- year long pre-clinical trial for patients with Alzheimer's disease, the most common adverse reactions were nausea and weight loss.[26][27][28] Reasons for using Resveratrol include: (1) small nanomolar levels can be

detected in cerebrospinal fluid; (2) they are safe and well tolerated; (3) changes in the biology of AD Trajectories of markers; (4) preserve the integrity of the blood-brain barrier, and (5) regulates the CNS immune response.

Several other studies have depicted that resveratrol has a neuroprotective effect in experimental models of Alzheimer's and Parkinson's, although due to its rapid metabolism and low bioavailability it limits its clinical application. It has also been suggested that structural changes to resveratrol molecules, including glycosylation, halogenation, alkylation, hydroxylation and methylation could lead to the buildout of derivatives with enhanced biological and pharmacological activities. [30][31][32]

CHAPTER 3: MOLECULAR DOCKING

Molecular docking is a valuable implement for evaluating the drug-target interaction of parent compounds in a cheap, fast and reliable way. Basically, the purpose of molecular docking is to use computational techniques to determine the orientation of the ligand-receptor complex. The ability of nucleic acids and protein molecules to interrelate with smaller molecules and form supramolecular complexes plays an important role in protein dynamics, which affects its biological activity. [12]

The purpose of docking is to identify the correct conformation of the ligand, in the binding pockets. The structure of the protein molecule, and predicts the affinity between the ligand and the receptor molecule. According to the type of ligand, molecular docking can be divided into :

- Protein-ligand docking
- Protein-nucleic acid docking
- Protein-protein docking[13]

The aspects of molecular docking approach can be utilised to model the interplay sbetween the protein and a ligand (compact molecules), at the atomic level and help us in deciphering the behaviour of the ligand in the binding site of the protein molecule.[2]

Therefore, docking model can be used to identify the novel drug-like molecules, many works have already been done, and it helps in the identification of better molecules than the ones already existing.

3.1 PRE-REQUISITES

Acetylcholinesterase, target ligand (drug and phytochemicals), Autodock 4.2.6, Open babel 3.1.1, MGL tools 1.5.6, Discovery Studio 2020, Proteinsplus (online tool for visualisation).

3.2 METHODOLOGY

1. The sequence of 4m0e (Structure of human acetylcholinesterase in complex with dihydrotanshinone I) was obtained from Protein data bank with Uniprot Id P22303.[18] The conformations of approved drug Rivastigmine (PubChem ID:77991) and phytochemicals Berberine (PubChem ID: 2353), Quercetin (PubChem ID: 5280343), Assoanine (PubChem ID: 443725), Oxoassoanine (PubChem ID: 321919), Vasconine (PubChem ID: 443690), Retusin (PubChem ID: 5352005), Huperzine A (PubChem ID: 854026) and Resveratrol (PubChem ID: 445154) were downloaded from PubChem.

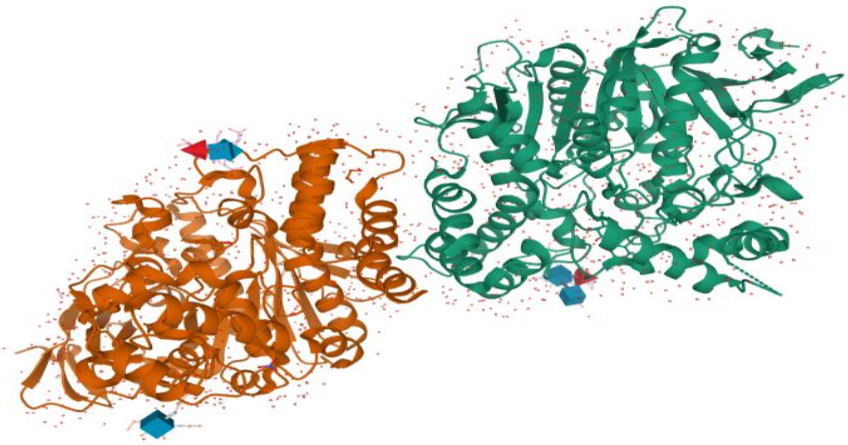
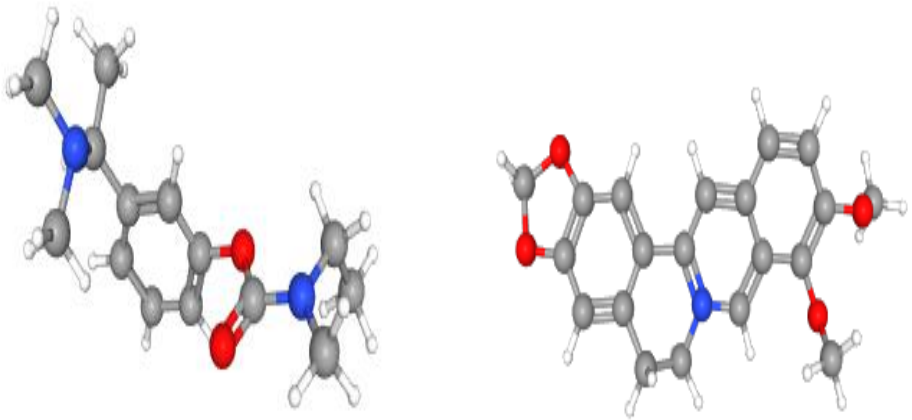
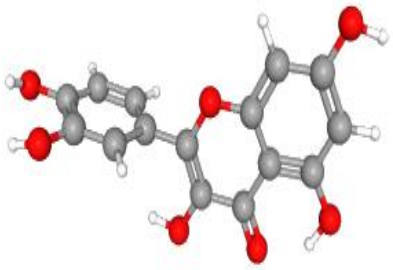


Fig 4: 3D Structure of human acetylcholinesterase in complex with dihydotanshinone I (RCSB ID: 4M0E)



(a) **(b)**



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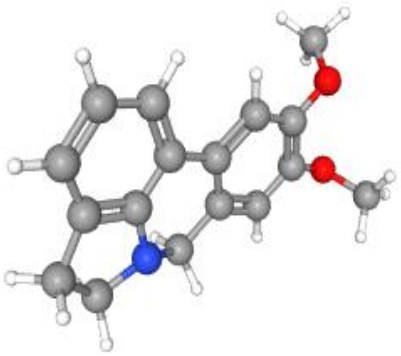

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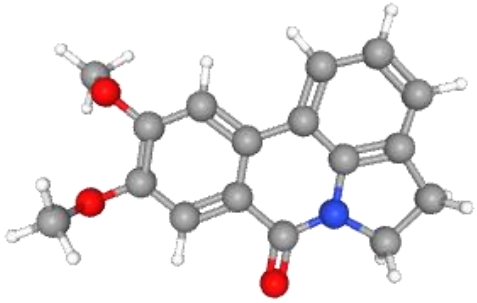

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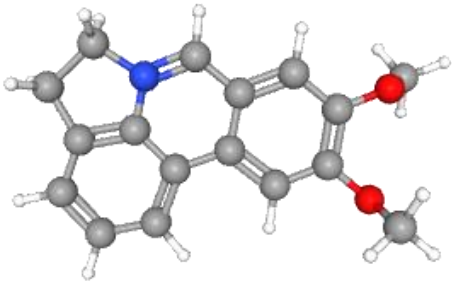

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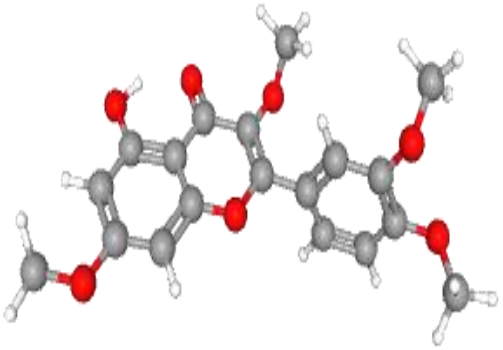

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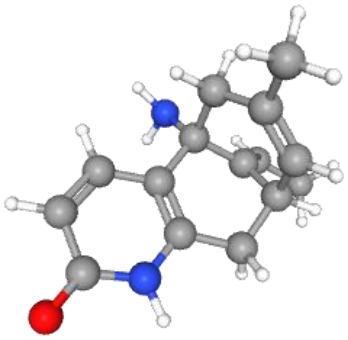

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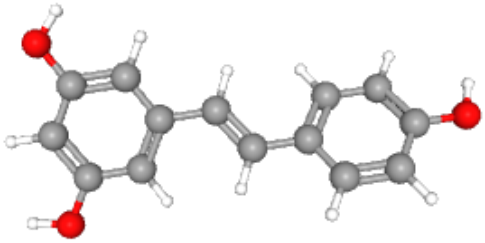


(i)

Fig 5: 3D structure of: (a) Rivastigmine (b) Berberine (c) Quercetin (d) Assoanine (e) Oxoassoanine (f) Vasconine (g) Retusin (h) Huperzine A (i) Resveratrol

2. Open Babel 3.1.1 was used to convert the ligand sequence from sdf format to pdb format.
3. Autodock tool was used to read the protein and ligand molecule.
4. Protein molecule was prepared by removing the extra chains, water molecules, balanced the molecule by adding polar hydrogens and adding Kollman Charges.
5. The ligand was prepared by choosing and detecting its root.
6. The active catalytic site of the protein molecule was highlighted (Ser203, Glu 334, His 447).
7. Grid box was set according to the coordinates (-2.984, -46.050, 25.794) for Rivastigmine, Berberine,Assoanine and Quercetin.
8. After execution of autogrid and autodock files, the obtained output file was analysed for the results.

CHAPTER 4: RESULTS & DISCUSSION

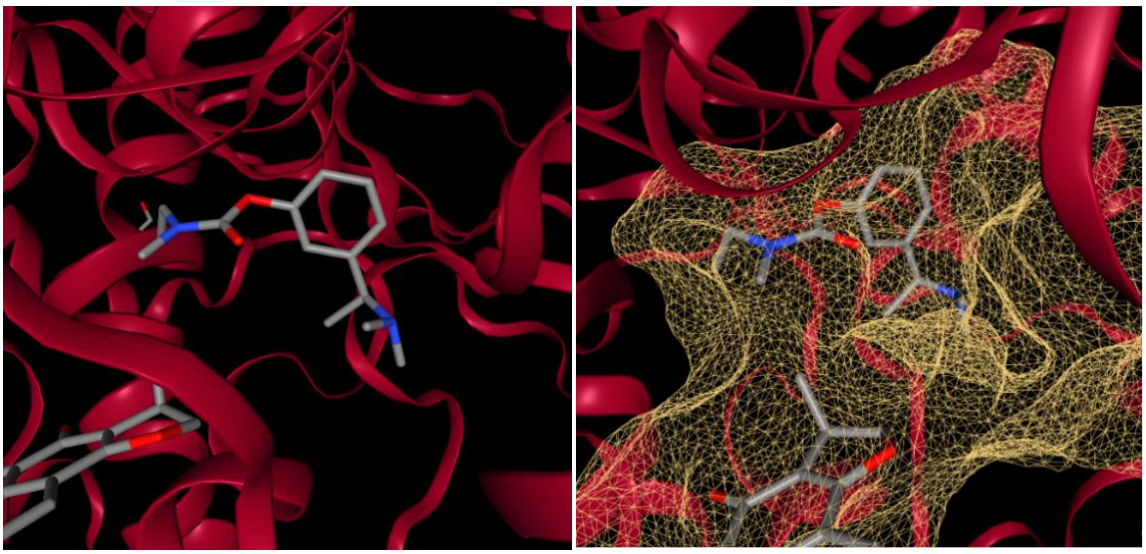
4.1 RIVASTIGMINE:

The result of generated output file is as follow:

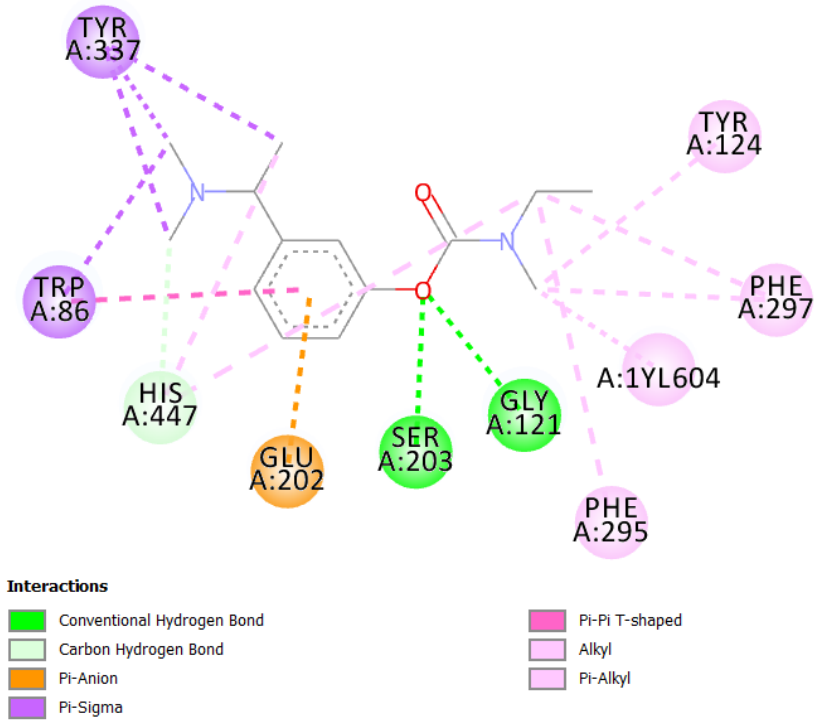
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	-5.38	0.00	53.06	RANKING
1	2	22	-5.32	1.92	52.73	RANKING
1	3	14	-5.02	1.87	52.84	RANKING
1	4	21	-5.00	0.37	52.99	RANKING
1	5	11	-4.94	1.83	52.81	RANKING
1	6	25	-4.12	1.88	52.71	RANKING
1	7	7	-3.61	1.73	52.85	RANKING
1	8	15	-0.24	1.10	52.42	RANKING
1	9	19	+4.26	1.90	53.45	RANKING
2	1	18	-4.31	0.00	52.75	RANKING
2	2	13	-3.58	1.97	51.38	RANKING
2	3	1	-3.52	1.91	51.46	RANKING
2	4	2	-3.47	1.94	51.95	RANKING
2	5	10	-3.08	1.95	52.22	RANKING
2	6	28	-3.05	1.91	51.45	RANKING
2	7	3	-2.89	1.18	51.81	RANKING
2	8	12	-0.66	1.78	51.55	RANKING
2	9	4	-0.45	1.50	51.65	RANKING
3	1	16	-3.80	0.00	51.99	RANKING
3	2	5	-3.53	1.66	51.99	RANKING
3	3	8	-3.50	1.67	51.93	RANKING
3	4	17	-3.21	1.88	51.25	RANKING
3	5	29	-3.18	1.88	51.43	RANKING
3	6	27	-1.49	2.00	51.84	RANKING
3	7	26	-1.28	1.83	51.83	RANKING
3	8	30	-1.12	1.80	51.98	RANKING
4	1	23	-2.33	0.00	51.84	RANKING
4	2	20	-1.64	1.02	51.94	RANKING
4	3	24	-0.81	0.77	51.53	RANKING
4	4	6	+0.51	1.75	52.90	RANKING

Table 1: RMSD Table for Rivastigmine (30 runs)

Run 9 has the lowest binding energy, therefore this conformation exhibited the best bonding with active sites of Acetylcholinesterase.



(a) **(b)**



(c)

Fig 6: (a)protein ligand (Rivastigmine) complex with best conformation with lowest binding energy

(b)Protein-ligand complex in binding pocket (c) 2D interaction of Rivastigmine with acetylcholinesterase

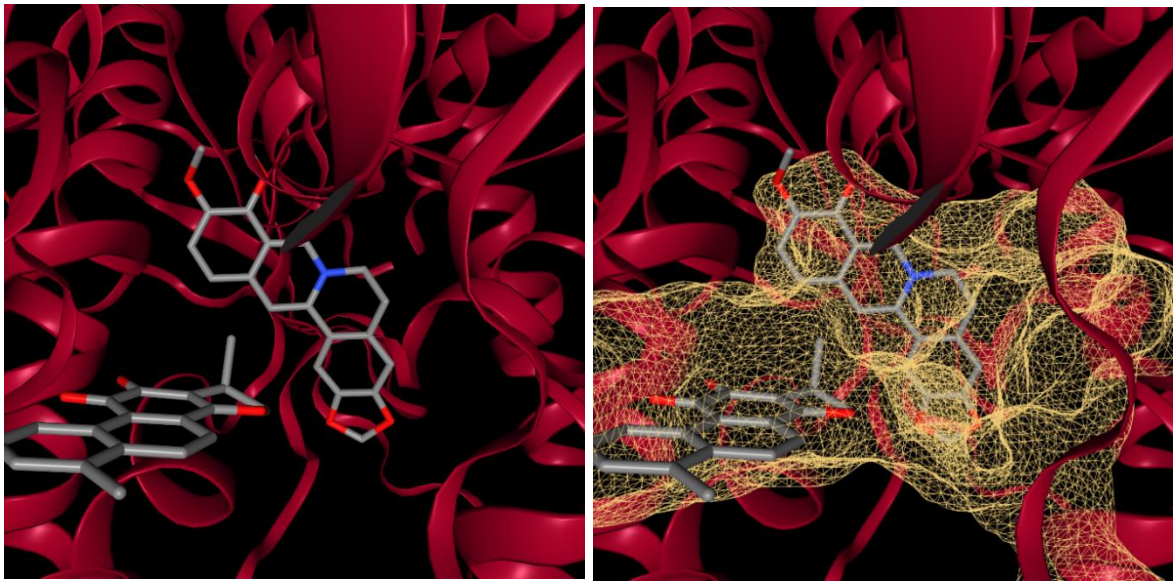
4.2 BERBERINE:

The output file generated is as follows:

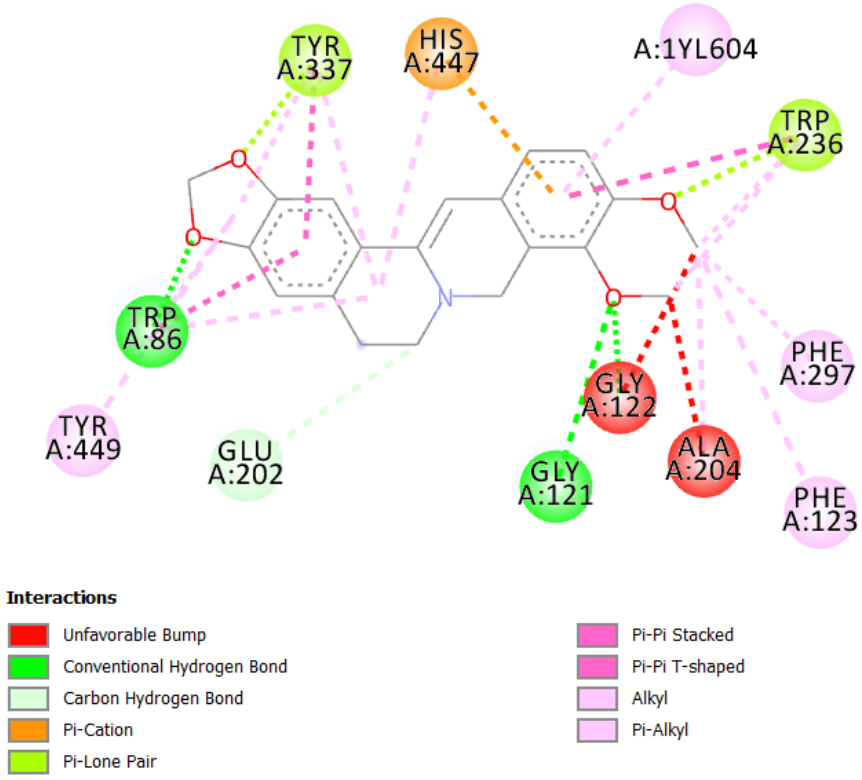
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	23	+34.92	0.00	51.92	RANKING
1	2	26	+36.09	0.06	51.94	RANKING
1	3	1	+36.10	0.04	51.89	RANKING
1	4	21	+36.12	0.04	51.90	RANKING
1	5	2	+36.21	0.05	51.90	RANKING
1	6	17	+36.26	0.07	51.86	RANKING
1	7	4	+36.58	0.06	51.92	RANKING
1	8	18	+36.83	0.06	51.91	RANKING
1	9	22	+36.84	0.13	51.92	RANKING
1	10	15	+37.05	0.06	51.90	RANKING
1	11	25	+37.06	0.07	51.88	RANKING
1	12	30	+37.22	0.12	51.88	RANKING
1	13	9	+37.22	0.11	51.89	RANKING
1	14	3	+37.38	0.09	51.87	RANKING
1	15	11	+37.56	0.16	51.86	RANKING
1	16	8	+37.62	0.19	51.86	RANKING
1	17	14	+38.38	0.14	51.98	RANKING
1	18	19	+39.16	0.20	51.91	RANKING
1	19	29	+40.57	0.24	51.98	RANKING
1	20	24	+44.82	1.63	53.93	RANKING
1	21	13	+44.93	1.63	53.95	RANKING
1	22	20	+45.04	1.62	53.95	RANKING
1	23	10	+45.16	1.62	53.94	RANKING
1	24	28	+45.23	1.62	53.93	RANKING
1	25	5	+46.46	1.61	53.91	RANKING
1	26	12	+50.26	0.55	52.08	RANKING
1	27	27	+50.79	1.61	53.95	RANKING
2	1	16	+56.39	0.00	54.15	RANKING
2	2	6	+63.40	0.28	54.22	RANKING
2	3	7	+68.34	0.65	53.67	RANKING

Table 2: RMSD Table for Berberine (30 runs)

Run 23 exhibited the lowest binding energy, with best conformation bound to acetylcholinesterase.



(a) **(b)**



(c)

Fig 7: (a)Protein-ligand (Berberine) complex exhibiting lowest binding energy (conformation 23)

(b)Protein ligand complex in binding pocket (c) 2D interaction of Berberine with acetylcholinesterase

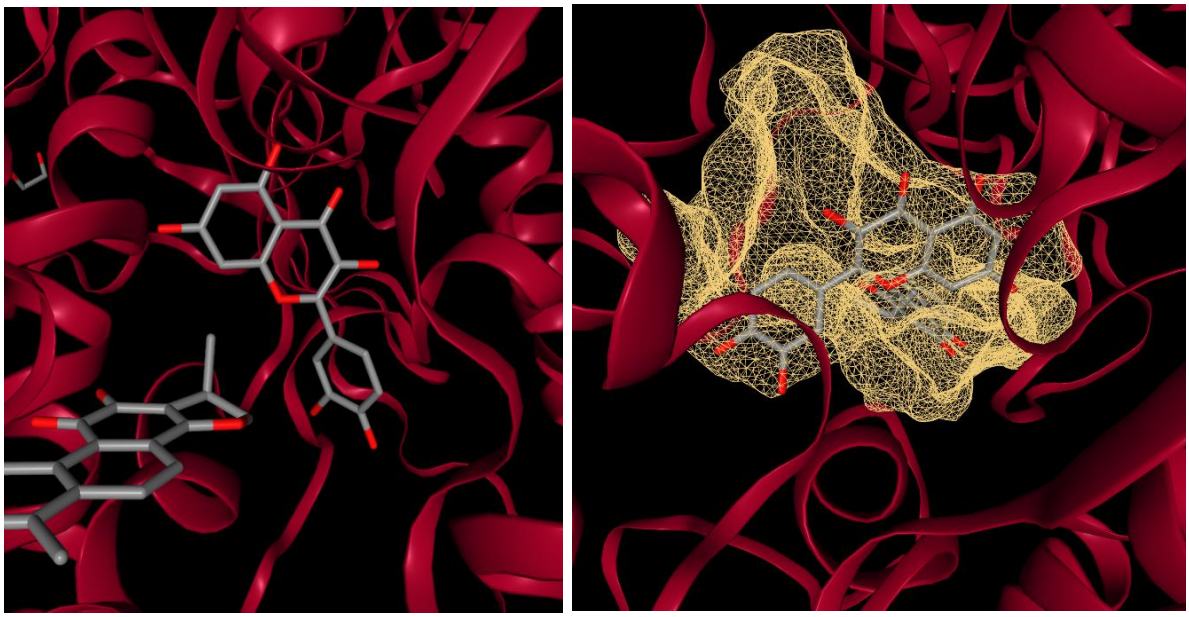
4.3 QUERCETIN:

The output file generated is as follows:

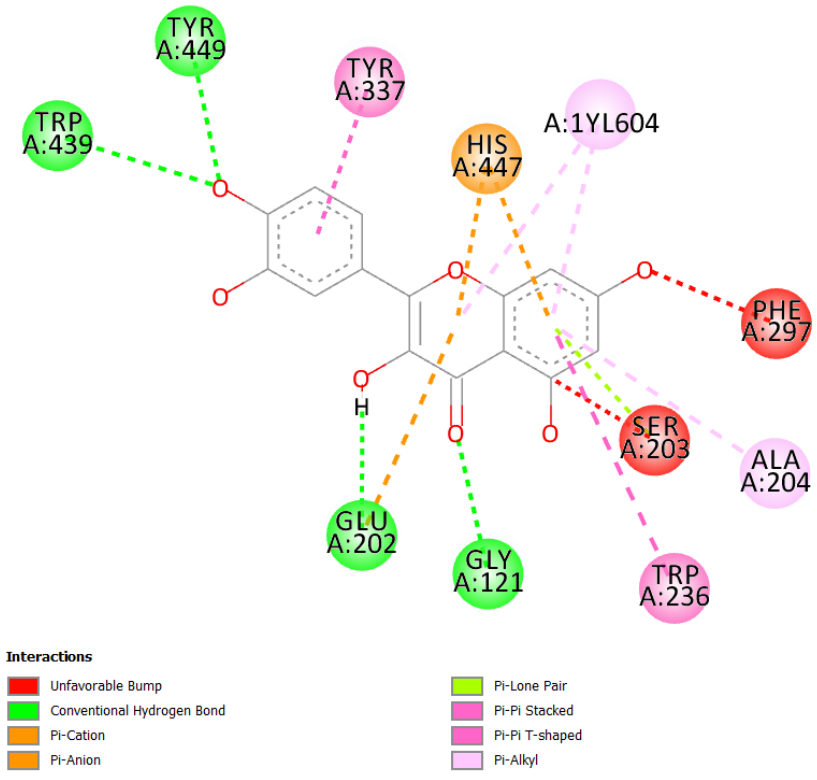
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	19	+10.52	0.00	52.02	RANKING
1	2	14	+10.61	1.32	53.36	RANKING
1	3	4	+10.71	1.27	53.32	RANKING
1	4	6	+11.20	0.55	52.39	RANKING
1	5	27	+11.21	0.32	52.18	RANKING
1	6	20	+11.39	1.27	53.27	RANKING
1	7	29	+12.25	1.27	53.32	RANKING
1	8	7	+17.32	0.50	51.73	RANKING
1	9	11	+17.65	1.42	53.28	RANKING
1	10	2	+20.88	1.63	50.78	RANKING
1	11	16	+22.99	1.73	52.62	RANKING
1	12	21	+23.03	1.73	52.64	RANKING
1	13	26	+23.14	1.73	52.63	RANKING
1	14	5	+24.33	1.64	52.57	RANKING
1	15	10	+31.31	1.79	53.11	RANKING
1	16	1	+38.60	1.21	51.65	RANKING
2	1	12	+14.49	0.00	50.89	RANKING
2	2	3	+14.80	0.09	50.92	RANKING
2	3	13	+15.22	0.53	51.00	RANKING
2	4	9	+15.23	0.53	51.01	RANKING
2	5	15	+15.33	0.52	51.00	RANKING
2	6	25	+15.70	0.39	51.05	RANKING
2	7	18	+15.95	0.41	51.12	RANKING
2	8	17	+15.97	0.52	51.07	RANKING
2	9	24	+16.07	0.52	51.09	RANKING
2	10	22	+16.22	0.42	51.10	RANKING
2	11	30	+16.47	0.42	51.09	RANKING
2	12	8	+21.01	1.22	51.06	RANKING
3	1	28	+20.26	0.00	54.57	RANKING
4	1	23	+93.27	0.00	47.03	RANKING

Table 3: RMSD table for Quercetin (30 runs)

During the 19th run, lowest binding energy was observed, this conformation had the best binding affinity for acetylcholinesterase.



(a) **(b)**



(c)

Fig 8: (a) Protein ligand (Quercetin) complex having the shown conformation and lowest binding energy. (b) Protein-ligand complex in the binding pocket (c) 2D interaction of Quercetin with acetylcholinesterase

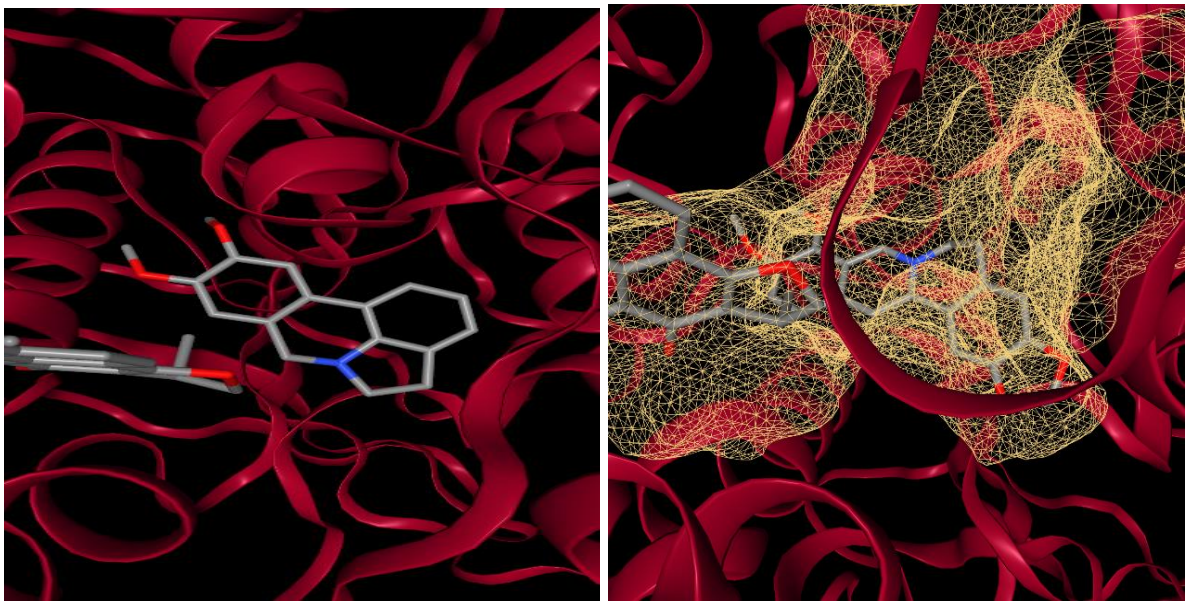
4.4 ASSOANINE:

The generated output file is as follows:

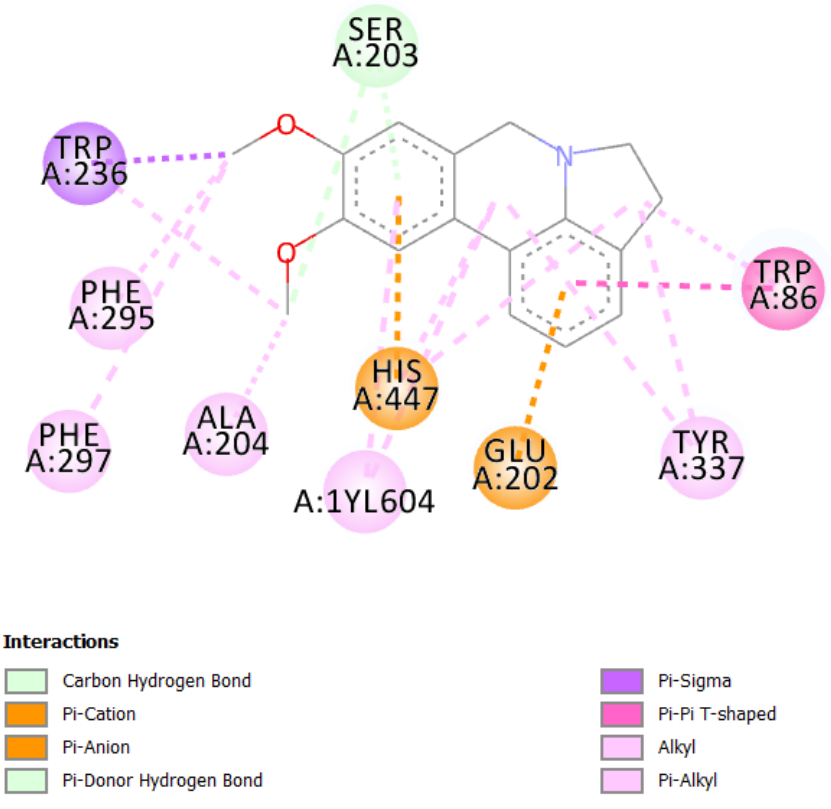
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	27	+3.86	0.00	51.55	RANKING
1	2	19	+4.27	1.26	52.07	RANKING
1	3	7	+4.33	0.99	51.99	RANKING
1	4	14	+4.35	1.01	52.02	RANKING
1	5	6	+4.35	1.01	52.02	RANKING
1	6	17	+4.40	1.02	52.04	RANKING
1	7	11	+4.41	1.33	51.63	RANKING
1	8	1	+4.41	0.99	52.01	RANKING
1	9	28	+4.41	0.99	52.00	RANKING
1	10	26	+4.43	1.02	52.02	RANKING
1	11	25	+4.46	1.30	51.65	RANKING
1	12	5	+4.46	1.25	52.07	RANKING
1	13	2	+4.47	1.26	52.11	RANKING
1	14	10	+4.56	1.22	52.23	RANKING
1	15	8	+4.65	1.20	52.21	RANKING
1	16	15	+4.66	1.20	52.20	RANKING
1	17	16	+4.70	1.00	52.00	RANKING
1	18	12	+4.71	1.22	52.25	RANKING
1	19	13	+4.71	1.20	52.23	RANKING
1	20	30	+4.72	1.01	52.01	RANKING
1	21	3	+4.75	1.25	52.28	RANKING
1	22	23	+4.82	1.02	52.04	RANKING
1	23	21	+6.21	1.98	52.02	RANKING
1	24	9	+6.23	1.98	51.97	RANKING
1	25	18	+6.25	1.98	52.02	RANKING
1	26	29	+6.30	1.97	52.01	RANKING
1	27	4	+6.43	1.96	51.97	RANKING
1	28	20	+6.46	1.96	51.97	RANKING
2	1	24	+13.89	0.00	54.88	RANKING
2	2	22	+14.01	0.02	54.87	RANKING

Table 4: RMSD table for Assoanine (30 runs)

In the case of molecular docking of Assoanine with Acetylcholine, run number 27 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 9: (a) Protein ligand (Assoanine) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Assoanine with acetylcholinesterase

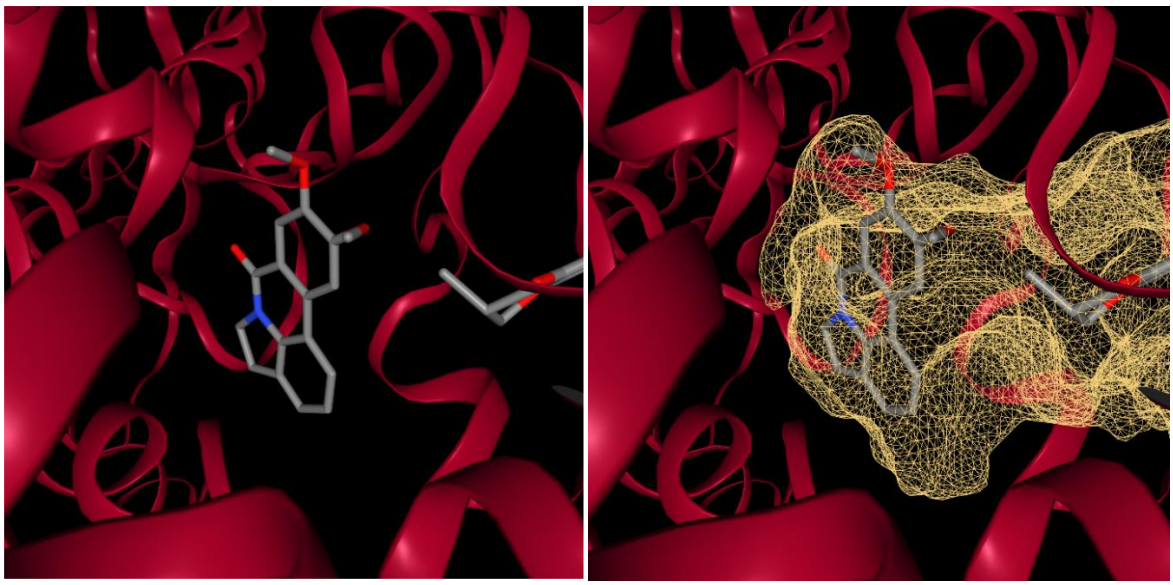
4.5 OXOASSOANINE:

The generated output file is as follows:

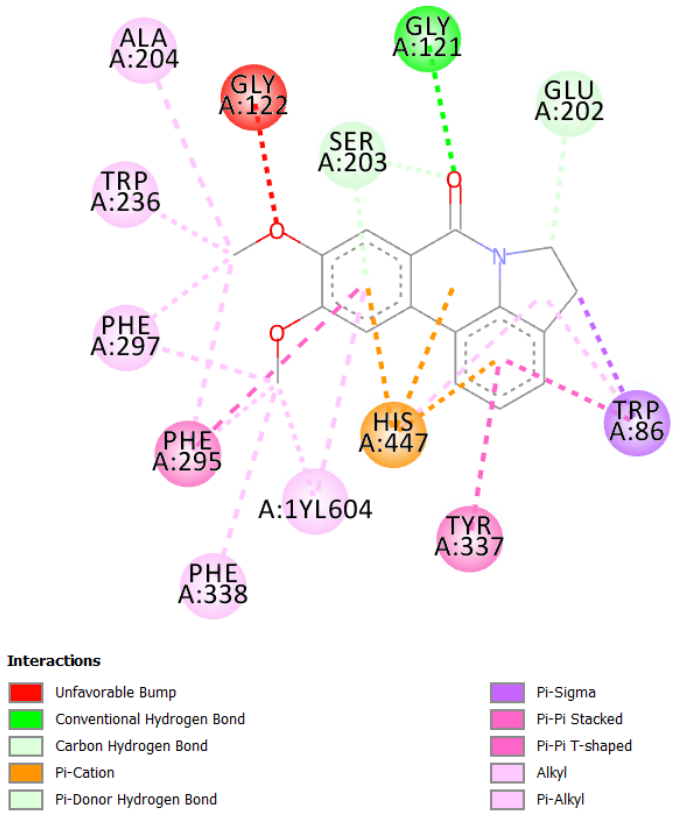
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	25	+17.10	0.00	51.89	RANKING
1	2	8	+17.21	0.04	51.89	RANKING
1	3	17	+17.40	1.35	51.54	RANKING
1	4	28	+17.40	0.37	51.78	RANKING
1	5	26	+17.44	0.14	51.86	RANKING
1	6	2	+17.59	0.42	51.72	RANKING
1	7	9	+17.60	0.20	51.88	RANKING
1	8	22	+17.66	0.11	51.83	RANKING
1	9	13	+17.69	0.11	51.85	RANKING
1	10	20	+17.78	0.14	51.86	RANKING
1	11	15	+17.81	1.35	51.50	RANKING
1	12	1	+17.83	0.44	51.71	RANKING
1	13	21	+17.95	0.28	51.89	RANKING
1	14	16	+18.25	0.53	51.63	RANKING
1	15	5	+19.78	1.35	51.49	RANKING
1	16	19	+21.75	0.53	51.62	RANKING
1	17	27	+23.57	0.49	51.77	RANKING
1	18	23	+24.11	1.77	52.07	RANKING
1	19	14	+24.41	1.79	52.02	RANKING
1	20	12	+24.45	1.78	52.02	RANKING
1	21	10	+24.56	1.76	52.07	RANKING
2	1	24	+23.35	0.00	52.06	RANKING
2	2	4	+23.79	0.05	52.06	RANKING
2	3	30	+23.96	0.06	52.06	RANKING
2	4	29	+24.11	0.07	52.06	RANKING
2	5	6	+24.45	0.10	52.05	RANKING
2	6	11	+25.62	0.45	51.90	RANKING
2	7	7	+27.89	0.41	51.95	RANKING
2	8	18	+28.45	0.81	51.49	RANKING
2	9	3	+29.59	0.87	51.52	RANKING

Table 5: RMSD table for Oxossoanine (30 runs)

In the case of molecular docking of Oxossoanine with Acetylcholine, run number 25 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 10: (a) Protein ligand (Oxoassoanine) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Oxoassoanine acetylcholinesterase

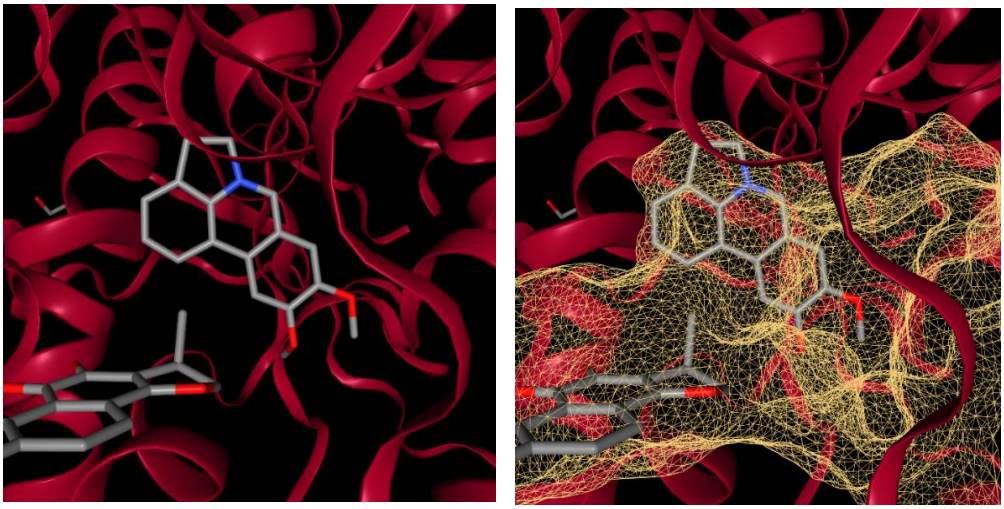
4.6 VASCONINE:

The generated output is as follows:

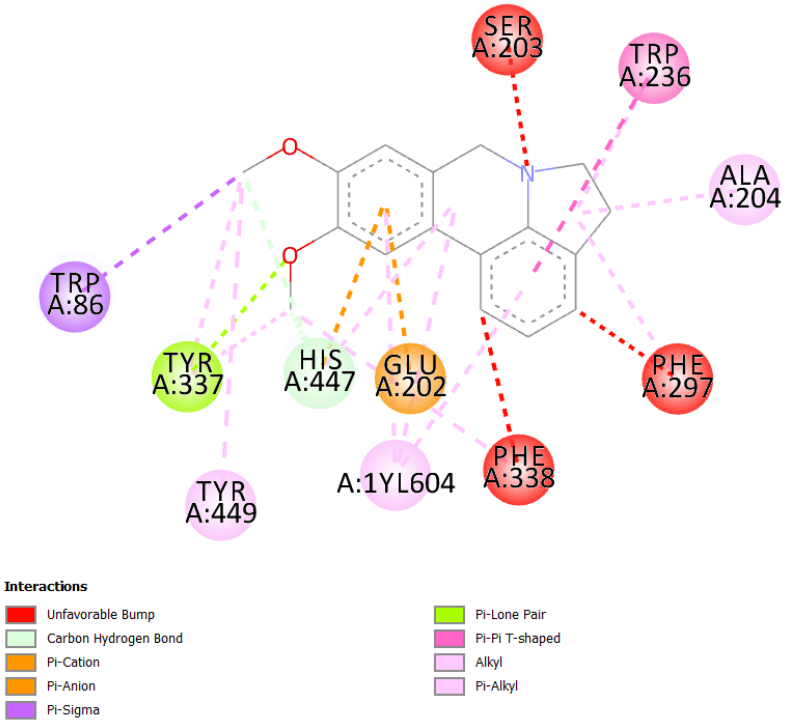
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	30	+39.27	0.00	51.05	RANKING
1	2	28	+39.73	0.38	51.35	RANKING
1	3	16	+39.79	0.07	51.01	RANKING
1	4	7	+40.25	0.29	51.29	RANKING
1	5	24	+40.59	0.33	51.31	RANKING
1	6	29	+41.45	0.21	50.96	RANKING
1	7	4	+66.21	1.00	51.58	RANKING
1	8	14	+79.83	1.01	50.83	RANKING
1	9	2	+87.40	0.93	51.31	RANKING
2	1	6	+48.49	0.00	49.92	RANKING
2	2	18	+51.25	0.23	49.81	RANKING
2	3	8	+51.77	0.21	49.81	RANKING
2	4	11	+52.19	0.18	49.80	RANKING
2	5	20	+55.56	0.94	50.11	RANKING
2	6	3	+56.69	0.18	49.97	RANKING
2	7	1	+58.81	1.05	50.23	RANKING
2	8	25	+65.23	1.31	50.46	RANKING
2	9	21	+67.54	1.63	50.76	RANKING
2	10	5	+68.44	1.35	50.73	RANKING
2	11	23	+71.44	1.54	51.65	RANKING
2	12	12	+72.66	1.22	50.52	RANKING
2	13	19	+73.74	1.35	51.18	RANKING
2	14	26	+75.04	1.59	50.86	RANKING
3	1	15	+69.08	0.00	53.75	RANKING
4	1	13	+76.40	0.00	53.57	RANKING
4	2	10	+77.85	0.11	53.59	RANKING
4	3	22	+78.61	0.34	53.77	RANKING
4	4	27	+87.48	0.93	53.93	RANKING
4	5	17	+87.76	0.92	53.90	RANKING
5	1	9	+150.75	0.00	48.04	RANKING

Table 6: RMSD table for Vasconine (30 runs)

In the case of molecular docking of Vasconine with Acetylcholine, run number 30 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 11: (a) Protein ligand (Vasconine) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Vasconine with acetylcholinesterase

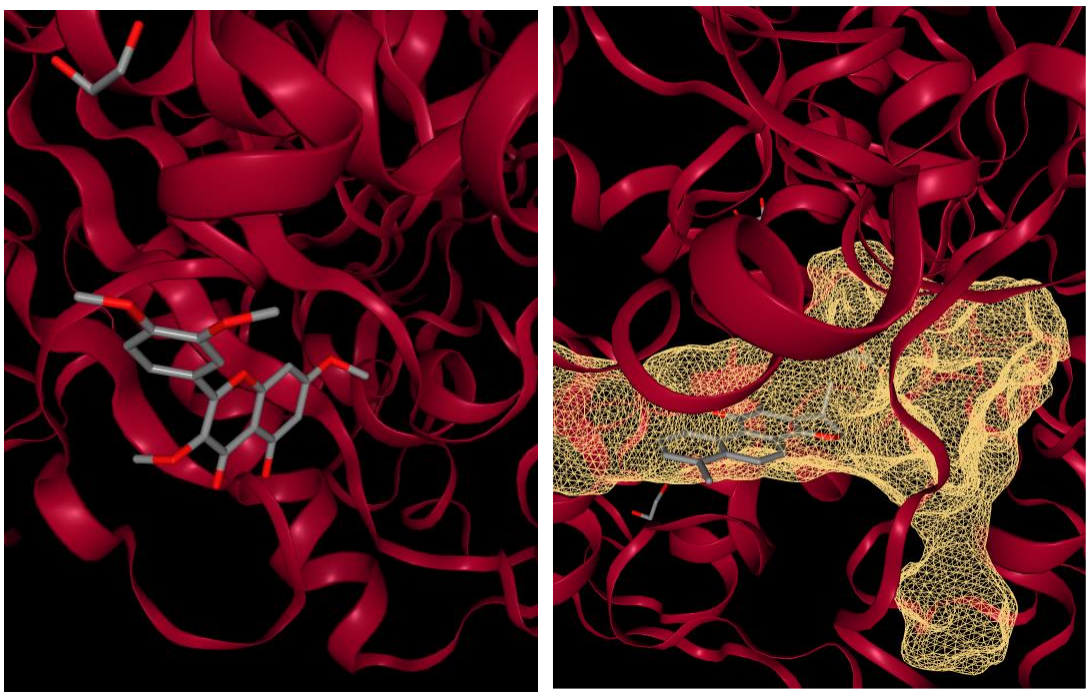
4.7 RETUSIN:

The generated output is as follows:

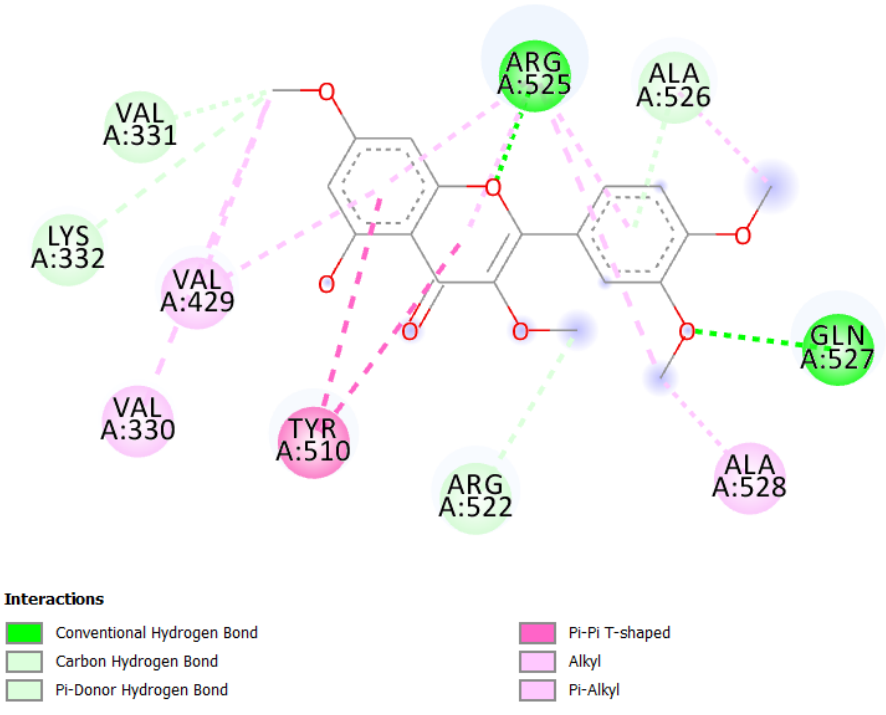
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	20	-6.33	0.00	56.29	RANKING
2	1	1	-6.09	0.00	54.78	RANKING
2	2	9	-5.19	1.60	55.31	RANKING
3	1	25	-5.95	0.00	38.57	RANKING
3	2	27	-5.59	1.61	39.45	RANKING
4	1	18	-5.56	0.00	63.93	RANKING
5	1	7	-5.52	0.00	37.85	RANKING
5	2	12	-5.36	1.37	37.71	RANKING
6	1	14	-5.39	0.00	60.29	RANKING
7	1	11	-5.35	0.00	36.90	RANKING
8	1	30	-5.34	0.00	47.28	RANKING
9	1	28	-5.24	0.00	56.85	RANKING
10	1	24	-5.22	0.00	69.05	RANKING
11	1	29	-5.19	0.00	37.31	RANKING
12	1	15	-5.14	0.00	68.95	RANKING
13	1	4	-4.99	0.00	55.46	RANKING
13	2	16	-4.78	0.49	55.32	RANKING
14	1	8	-4.95	0.00	37.43	RANKING
15	1	2	-4.89	0.00	38.08	RANKING
16	1	13	-4.77	0.00	38.29	RANKING
17	1	6	-4.77	0.00	65.34	RANKING
18	1	26	-4.68	0.00	72.79	RANKING
19	1	23	-4.65	0.00	36.84	RANKING
20	1	3	-4.59	0.00	57.63	RANKING
21	1	5	-4.54	0.00	34.89	RANKING
22	1	22	-4.35	0.00	41.73	RANKING
23	1	10	-4.35	0.00	43.28	RANKING
24	1	19	-4.16	0.00	76.47	RANKING
25	1	17	-4.13	0.00	61.53	RANKING
26	1	21	-3.97	0.00	50.13	RANKING

Table 7: RMSD table for Retusin (30 runs)

In the case of molecular docking of Retusin with Acetylcholine, run number 20 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 12: (a) Protein ligand (Retusin) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Retusin wth acetylcholinesterase

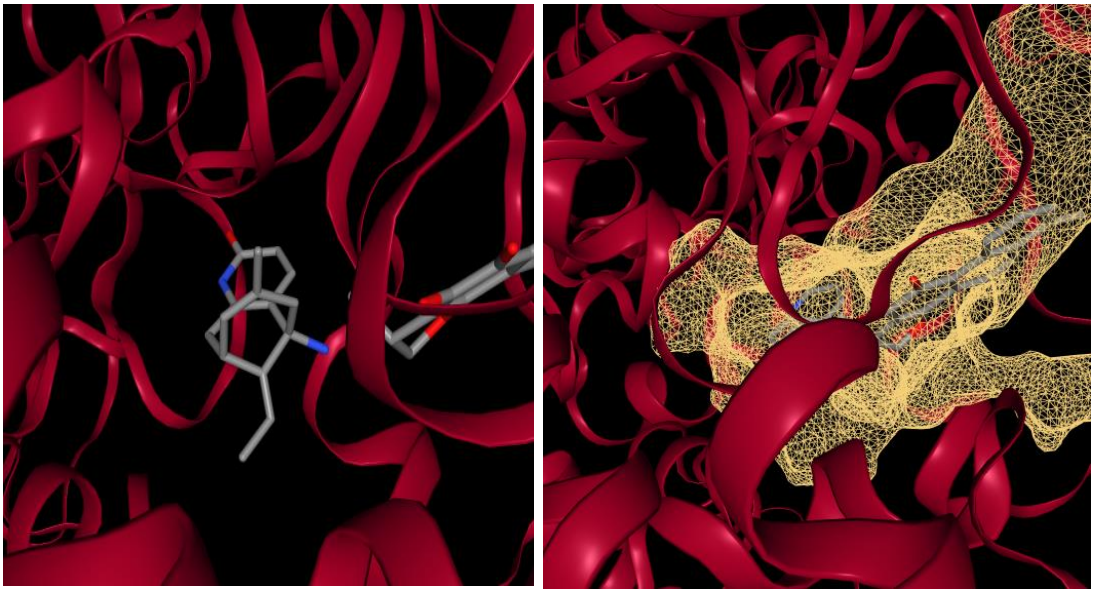
4.8 HUPERZINE A:

The generated output is a s follows:

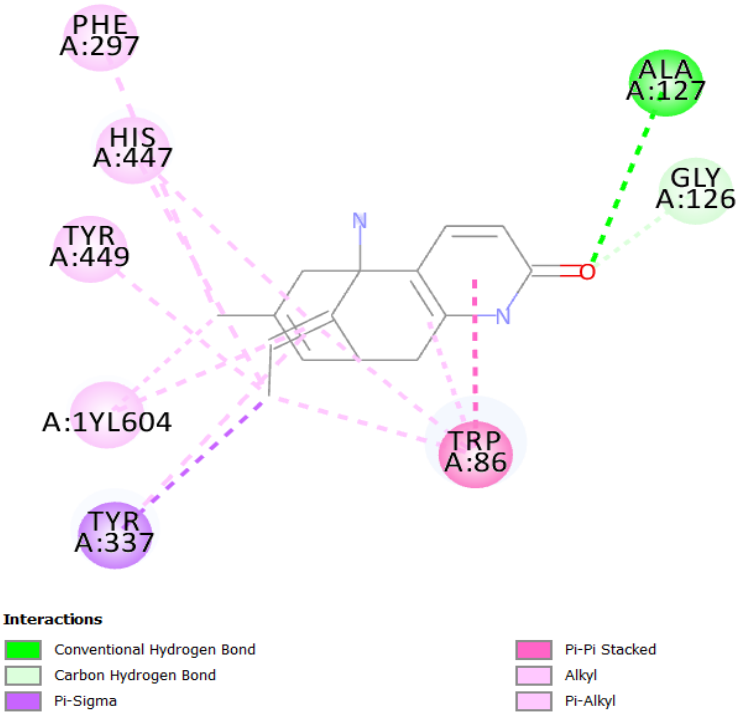
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	10	-8.85	0.00	53.58	RANKING
1	2	17	-8.82	0.13	53.56	RANKING
1	3	18	-7.71	0.43	53.48	RANKING
2	1	2	-8.03	0.00	39.48	RANKING
2	2	28	-8.03	0.03	39.46	RANKING
2	3	4	-8.03	0.07	39.46	RANKING
2	4	22	-7.93	0.19	39.49	RANKING
2	5	13	-7.87	0.62	39.42	RANKING
2	6	25	-7.85	0.60	39.42	RANKING
2	7	11	-7.82	0.54	39.35	RANKING
2	8	21	-7.36	0.93	39.28	RANKING
3	1	3	-7.97	0.00	49.80	RANKING
4	1	20	-7.70	0.00	38.19	RANKING
4	2	12	-7.66	0.13	38.21	RANKING
4	3	24	-7.63	0.77	37.69	RANKING
4	4	23	-7.63	0.36	38.09	RANKING
5	1	16	-7.66	0.00	37.99	RANKING
6	1	19	-7.58	0.00	39.32	RANKING
6	2	8	-7.57	0.09	39.35	RANKING
6	3	26	-7.55	0.32	39.48	RANKING
7	1	29	-7.02	0.00	64.27	RANKING
7	2	5	-7.01	0.05	64.25	RANKING
7	3	1	-7.00	0.03	64.27	RANKING
8	1	9	-6.71	0.00	46.06	RANKING
8	2	14	-6.66	1.18	45.73	RANKING
9	1	27	-6.54	0.00	45.28	RANKING
10	1	7	-6.52	0.00	49.63	RANKING
11	1	15	-6.34	0.00	57.41	RANKING
12	1	6	-6.22	0.00	38.54	RANKING
13	1	30	-5.76	0.00	47.90	RANKING

Table 8: RMSD table for Huperzine A (30 runs)

In the case of molecular docking of Huperzine A with Acetylcholine, run number 10 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 13: (a) Protein ligand (Huperzine A) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Huperzine A with acetylcholinesterase

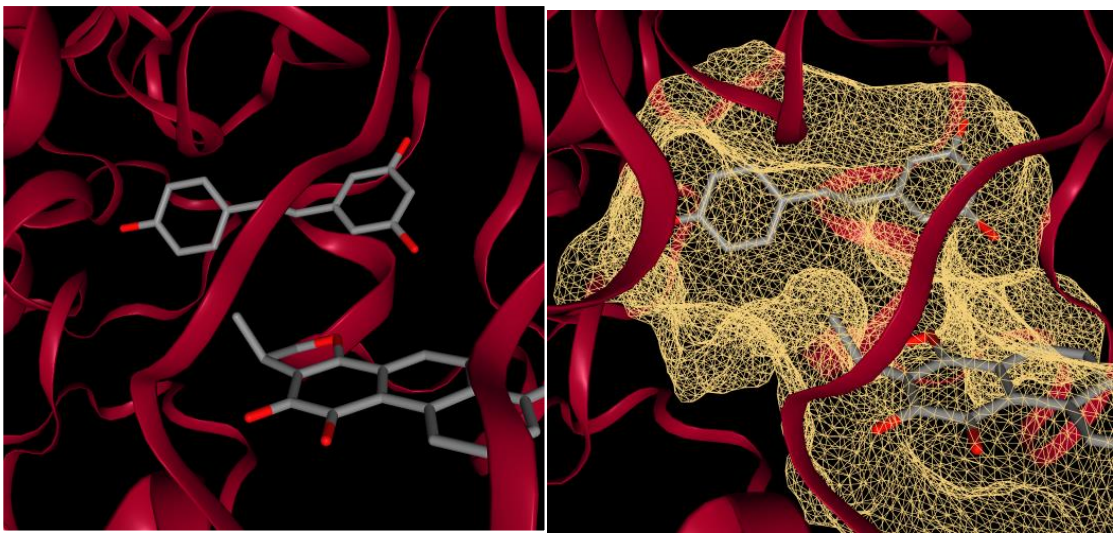
4.9 RESVERATROL:

The generated output is as follows:

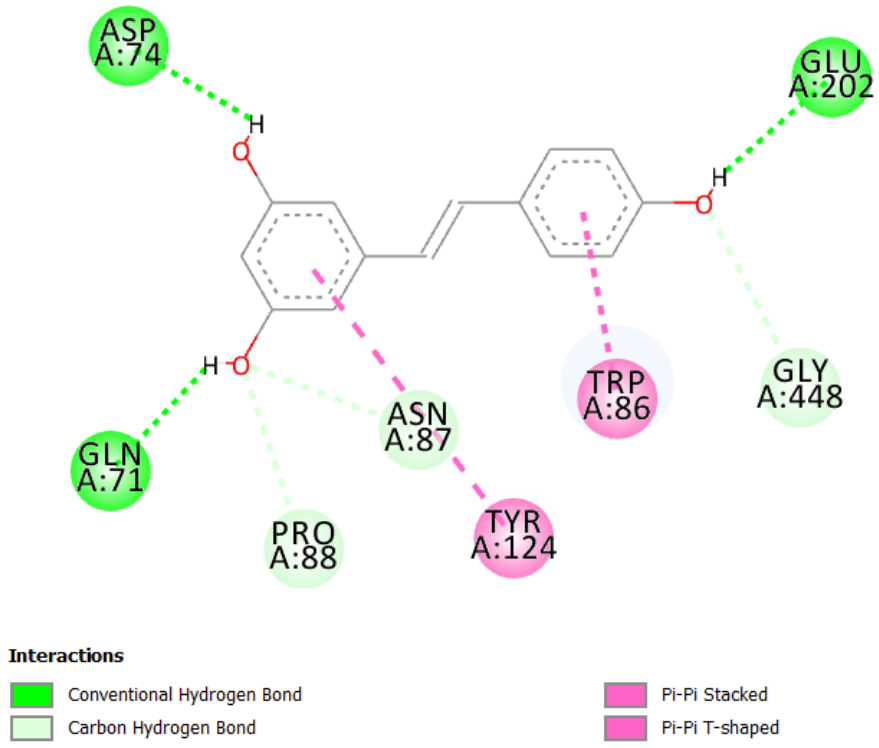
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	25	-7.60	0.00	53.82	RANKING
2	1	14	-5.68	0.00	37.13	RANKING
2	2	11	-5.15	1.33	37.63	RANKING
2	3	10	-4.84	1.83	37.75	RANKING
3	1	12	-5.51	0.00	53.43	RANKING
4	1	4	-5.44	0.00	64.22	RANKING
4	2	28	-4.84	1.83	64.41	RANKING
5	1	16	-5.43	0.00	48.27	RANKING
5	2	30	-4.74	0.78	48.32	RANKING
6	1	26	-5.39	0.00	51.13	RANKING
6	2	6	-5.31	0.45	51.38	RANKING
6	3	20	-4.83	0.91	51.38	RANKING
6	4	2	-4.71	0.95	50.76	RANKING
7	1	15	-5.38	0.00	39.64	RANKING
7	2	17	-5.34	0.29	39.60	RANKING
7	3	18	-5.33	0.62	39.51	RANKING
7	4	19	-5.24	0.85	39.21	RANKING
7	5	13	-5.22	1.68	39.91	RANKING
7	6	9	-5.12	1.58	39.89	RANKING
8	1	5	-5.33	0.00	39.06	RANKING
9	1	27	-5.20	0.00	64.96	RANKING
10	1	7	-5.08	0.00	38.38	RANKING
11	1	8	-4.93	0.00	67.65	RANKING
12	1	21	-4.71	0.00	57.10	RANKING
13	1	23	-4.61	0.00	39.21	RANKING
14	1	1	-4.58	0.00	56.59	RANKING
15	1	22	-4.28	0.00	39.58	RANKING
16	1	29	-4.22	0.00	69.20	RANKING
17	1	3	-4.09	0.00	61.71	RANKING
18	1	24	-4.00	0.00	50.58	RANKING

Table 9: RMSD table for Resveratrol (30 runs)

In the case of molecular docking of Resveratrol with Acetylcholine, run number 25 exhibited the lowest binding energy, and simultaneously best binding with it.



(a) **(b)**



(c)

Fig 14: (a) Protein ligand (Resveratrol) complex showing the best conformation having lowest binding energy. (b) Protein-ligand complex in binding pocket (c) 2D interaction of Resveratrol with acetylcholinesterase

The RMSD tables of these phytochemicals and drug suggests that Rivastigmine (considered as standard) is indeed a good inhibitor of Acetylcholinesterase while, Huperzine A followed by Resveratrol, Retusin and Assoanine show promising results while Quercetin, Oxoassoanine, Berberine and Vasconine can be considered as not so god potential acetylcholinesterase inhibitors (AChEi).

The binding energies of some of the investigated phytochemicals came out to be positive, indicating that they may have bonded in an unstable manner.

CHAPTER 5: CONCLUSION

The phytochemicals berberine, quercetin, assoanine and derivatives (oxoassoanine and vasconine), retusin, huperzine A and resveratrol were docked against our target enzyme: acetylcholinesterase. Rivastigmine, retusin, huperzine A and resveratrol exhibited negative binding energies with acetylcholinesterase, indicating stable binding with it and therefore acts to inhibit the activity of acetylcholinesterase. While Berberine, Quercetin, Assoanine and its derivatives exhibited positive binding energies, i.e. it bounds to acetylcholinesterase molecule but was unstable while inhibiting the molecule.

The best/lowest binding energies obtained were Huperzine A (-8.85 kcal/mol), Resveratrol (-7.60 kcal/mol), Retusin (-6.33 kcal/mol) in comparison to Rivastigmine (-5.38 kcal/mol). Owing to this we conclude that Huperzine A and Resveratrol can serve as promising potential acetylcholinesterase inhibitors, compared to other tested phytochemicals.

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