Identification of Compounds from Nigella *Sativa* as Potential drug for Alzheimer Disease: Bioinformatics Approach

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

Dedicated to

my beloved Parents and Siblings

Acknowledgement

praise and gratitude to **Almighty ALLAH**, the Omnipotent, the All-Aware, the most merciful, who showered upon me His blessings throughout the thick and thin of my life and who blessed me courage, good health, company and support of good teachers and friends to conceptualize, develop and complete my research. Peace and blessings of Allah be upon His dearest **Holy Prophet, Hazrat Muhammad (PBUH)** who exhorted his followers to seek knowledge from cradle to grave.

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Sania Rahman

Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disorder effecting 50 million people worldwide. Nigella *sativa* has been considered the best therapeutic herb among different cultures for decades for multiple antiviral, anticancer and analgesic properties.

Objectives: Our study aims to investigate the possible interaction of tau protein with Nigella *sativa* derivatives by implying various bioinformatic tools and to identify the potential compound from Nigella *sativa* that may help in drug discovery.

Methodology: In the present study, the technique molecular docking was implied using Molecular Operating Environment software (MOE) and PyRx to identify and potential inhibitor of tau protein present in Nigella *sativa* on the basis of their docking score. Moreover, the drug likeness of these compounds was predicted using Lipinski rule of five. The ligands interaction with tau protein were studied through LIGPLOT.

Results: (-5.04495 Kcal/mol) was obtained by the interaction of Dithymoquinone and 2mz7 in Molecular Operating Environment, nearest to the Galantamine **(-5.6872 Kcal/mol**) and **(-6.1 kcal/mol)** was obtained using PyRx highest score among the synthetic and natural compounds. Dithymoquinone seemed to have the capacity as the potential inhibitor against tau phosphorylation.

Conclusion: our study provides a useful insight for designing more effective and selective inhibitors for the treatment of Alzheimer disease.

Key words: Alzheimer disease, Nigella *Sativa*, Dithymoquinone, molecular docking, Molecular Operating Environment.

Table of Content

List of Figures

List of Tables

Introduction

Alzheimer is a neurodegenerative disease cause in elder populations effecting 15 million of population around the globe. Each year the number of patients increases by 0.5% in population of age 65 and rate is much higher in the population of age 85 and above i.e., 8%. AD survival rate is unusual(Evans et al., 1989). Impaired memory is the most common and early symptom of the disease. With the progression of disease cognitive and psychiatric symptoms starts to appear. Difficulty in recalling names, forming a new memory, taking time to name the things, and performing daily tasks with disturbance are prominent during early stages while impaired verbal and visual abilities are the signs and symptoms of severe AD. Delusion, anxiety, agitation, depression are the behavioural symptoms linked with the AD.

AD is a typical neurodegenerative disorder. While presenting with a clinically recognizable syndrome, the disease-defining capabilities are pathological. Microscopic neuropathological examination of the brain shows deposits of extracellular -amyloid protein in diffuse and Neuritic plaques. Intracellular adjustments include deposits of abnormally hyper-phosphorylated tau protein within the shape of neurofibrillary tangles, and there is significant loss of neuronal synapses. Additional functions encompass different neuropil pathology (e.g., neuropil threads), mobile pathology (e.g., granulovacuolar degeneration in the hippocampus), and regional mobile losses (mainly in the hippocampus). These pathological hallmarks are significant. Genetic and environmental impacts have been explained. But nevertheless, the fundamental mechanisms responsible for the improvement of this ailment are unknown. A form of pharmacologic interventions is available to improve the signs and symptoms of the ailment. These medications encompass cholinesterase inhibitors, which increase vital “cholinergic level” and ameliorate secondary effects of the disease. However, there are currently no therapies verified to have an effect on the course of this disease.

Many molecular and cellular changes occur within the brain of Alzheimer’s patient which can be observed during autopsy after death. Two definite reasons behind the disease are senile plaques and the Neuro fibrillary tangles.

The beta-amyloid protein is present in the brain and is from by the breakdown of amyloid precursor protein. In the Alzheimer’s brain, this protein clump collectively to form plaques and

disrupt cell feature. Research is ongoing to recognize how, and at what stage of the disease, the numerous varieties of beta-amyloid have an impact on Alzheimer's.

Neurofibrillary tangles are atypical accumulations of a protein known as tau that collect inside neurons. Healthy neurons, in part, are supported internally by using systems referred to as microtubules, which help guide nutrients and molecules from the cell to the axon and dendrites. In neurons, tau generally binds to and stabilizes microtubules. In Alzheimer's disease, however, ordinary chemical changes reason tau to detach from microtubules and attached with other tau molecules, forming threads that finally form tangles in the neurons. These tangles block the neuron's delivery machine, which effect the exchange of neurotransmitters between neurons.

Research revealed that Alzheimer's-associated mind modifications may additionally end result from a complicated interplay among extraordinary tau and beta-amyloid proteins and numerous different elements. Beta-amyloid clumps into plaques between neurons. As the level of beta-amyloid reaches a tipping factor, there is a rapid spread of tau all through the mind.

Most sufferers with signs and symptoms of dementia were presented to the general physician (Small et al., 1997). In 90% cases psychiatric and clinical tests are enough to diagnose AD Rasmusson et al., 1996).The most important diagnostic sources are the medical assessment and family member who could provide the information about the behavioural and psychiatric changes Small et al., 1997). Information of AD history in family is also useful. In case of genetic APOE 4 on the chromosome nineteen also help in the evaluation of disease. (Corder et al., 1993). Cognitive feature can be observed through different diagnoses, inclusive of principal hopelessness, tension disease, and dementia due to any other reason except for Alzheimer's, Alzheimer's sickness presents with cognitive symptoms. However, clinicians are cautioned to manage dementia screening devices to all older sufferers with memory or different cognitive problems (1994) .

At present, no treatment can reverse or cure the Alzheimer's. Therapies like cholinesterase inhibitors are useful in delaying symptom (Brookmeyer et al., 1998). Older sufferers metabolize tablets extra slowly and are extra at risk of damaging effects; there may be additional possibility of taking multiple medicines, heightening the chance of drug-drug interactions (Small et al., 1997). The primary goal of treatment for Alzheimer's disease is to improve patient lifestyle. Providing the affected person with great and stimulating sports,

inclusive of leisure therapy or pet remedy, can adequately assist on this goal. Three On the alternative hand, a few psychosocial therapies, specifically cognition-oriented treatments, can provoke frustration in demented sufferers and should be prevented. FDA approved four drugs for the treatment of Alzheimer's are memantine, donepezil, galantamine, and rivastigmine, all of which are cholinesterase inhibitors. Although the precise mechanism isn't understood clearly, each improves cholinergic neurotransmission by using preventing the breakdown of acetylcholine. Deficits of acetylcholine, a cholinergic neurotransmitter, were observed inside the brains of sufferers with Alzheimer's disease, suggesting that this neurotransmitter performs a function in cognition and memory. Though the cholinesterase inhibitors can produce modest improvements in cognition, their facet consequences include nausea, diarrhoea, and vomiting.

Literature review

2.1 Overview

Alzheimer is a degenerative brain disorder of unknown etiology and the most common form of dementia that usually starts in the late middle age or older age results in a progressive memory loss impaired thinking and personality disorder (Masters et al., 2015). AD must be considered a public crisis because today over 5 million adults are living with AD including 200,000 under the age of 65 and it is increasing in alarming rate ("2010 Alzheimer's Disease Facts and Figures.," 2010). Diagnosis can only be done after death by linking brain cells in an autopsy with biomarker however cerebrospinal fluid examination can be helpful for better prediction (Weller & Budson, 2018). Currently no potent therapy or medicine is available to cure AD however research for early detection and effective treatment is ongoing.

2.2 History

A German Neurologist name Alois Alzheimer in 1960 discovered AD by observing Auguste D. and elder women of 51 years have had impaired memory and disturbed personality (Z S Khachaturian & Radebaugh, 2019). She had an aggressive type of dementia (Hippius & Neundörfer, 2003). Dr Alois look after her for 5 years and observed several abnormalities including speech problem, confusion, and anxiety. After her death in 1906 an autopsy was performed by Dr Alois which revealed severe shrinkage of cerebral cortex (Wimo & Prince, 2010). Senile plaques and Neurofibrillary tangles were observed and considered as indication of AD (Hippius & Neundörfer, 2003).

2.3 Aging vs AD vs Dementia

Aging is normally confused with the Dementia and AD. Dementia and Aging are not same. Normal aging does not cause severe memory loss of decline in cognitive behaviour but other biological condition like weight loss, hair loss, weak senses, poor vision and hearing ability, fragile bones and metabolism may be the cause of healthy aging.

Dementia is characterized as gradual decrease of behavioural and psychiatric functional abilities enough to interfere in social performance in daily life (UK, 2007). Brain damage due to several disease can cause the Dementia. Dementia has different type with different cause and symptoms associated with respective type. For example HIV associated Dementia cause by the spread of HIV virus to the brain (Kaul et al., 2001) and frontotemporal Dementia affect

the temporal and frontal lobes of the brain (Young et al., 2018). However most common type of dementia is Alzheimer that is cause by the accumulation of beta Amyloid in the brain cells.

2.4 Presentation of Alzheimer's disease

AD is categorized into three major stages based on the symptoms. Medical Doctor can predict the stages by evaluating the symptoms and proposed the possible therapy. AD stages with their challenges are described in the figure 1 (Herndon, J. (2019). Figure 2 (Kumar, N. (2018) demonstrates the comparison of the healthy brain with the mild and the severe Alzheimer's.

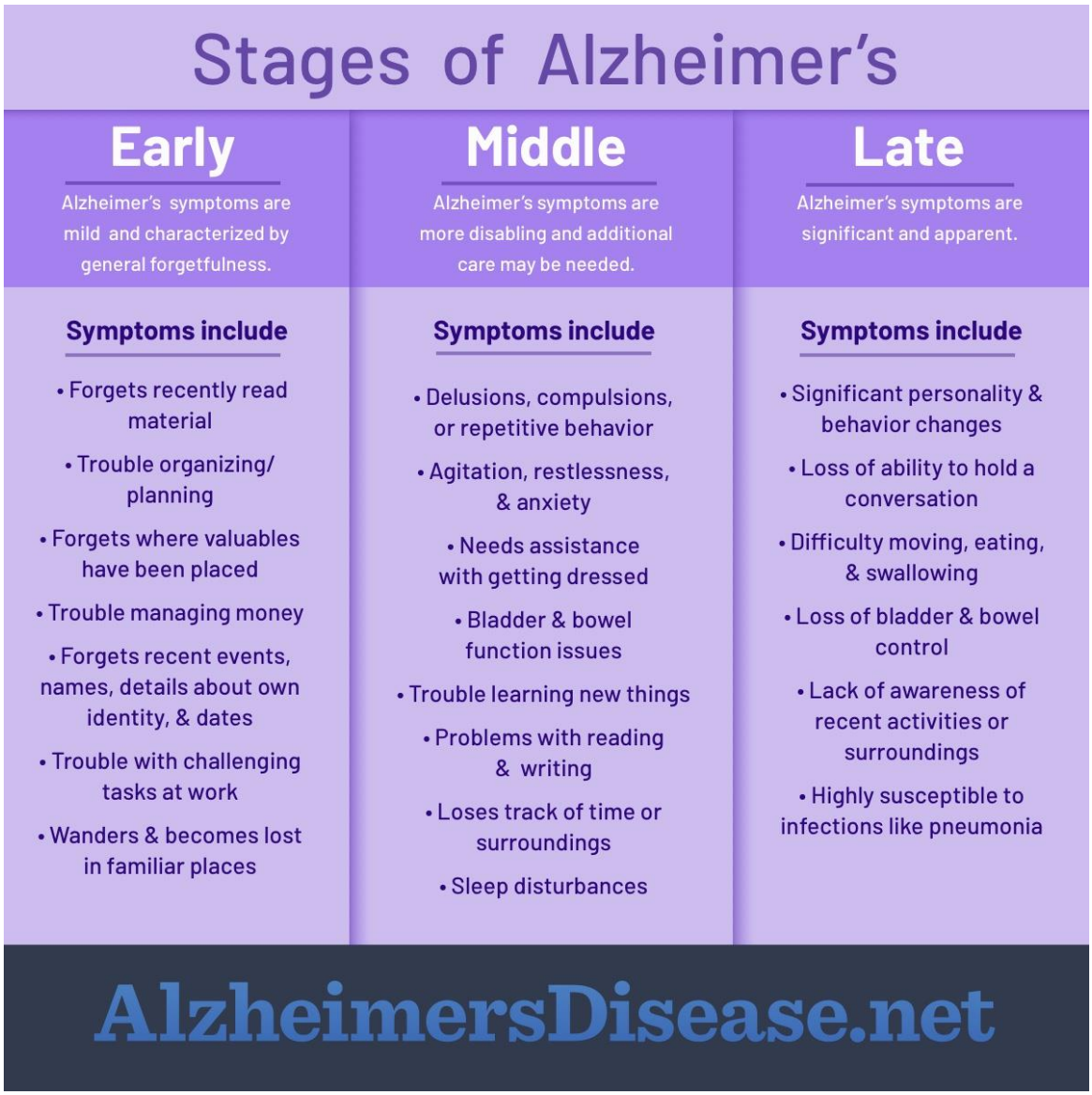


Figure 1: This figure demonstrates the stages and symptoms of Alzheimer's disease.

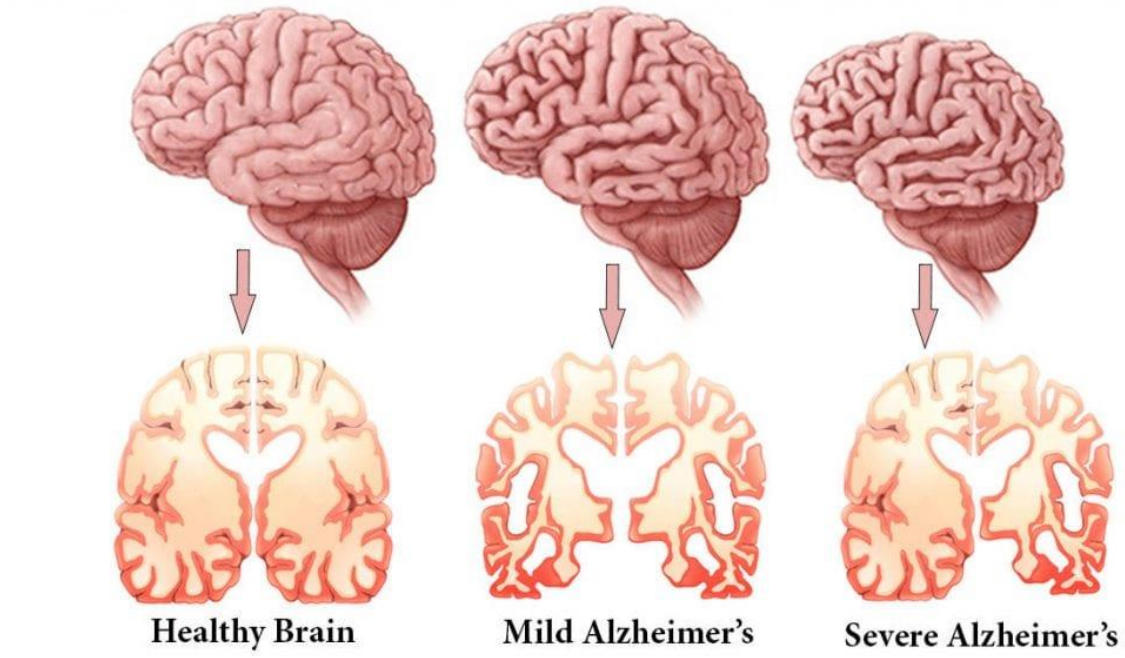


Figure 2: This figure demonstrates the physiological changes occurs in the brain from healthy to severe condition in Alzheimer's disease. (Kumar, N. (2018)

2.5 Deformities in Alzheimer's Patient Brain

Senile plaques and NFTs are the prominent deformities cause by AD in human brain. Neuritic and diffuse plaques are the types of plaques that are accumulated in the neurocortex of the brain around the Amyloid protein (Z S Khachaturian & Radebaugh, 2019) . NFTs are present in the cytoplasm of neurons in the entorhinal cortex.

Healthy neurons gradually loss its function with the progression of plaques and NFTs. Shrinkage of brain is followed by the death of neurons in the hippocampus that also restricts the ability of forming new memories in the patient.

2.6 Mortality

Mortality rate of AD rises noticeably since 1991. The primary cause of death is still unclear but the complications come with the severity of disease such as swallowing difficulty and loss of bowel and bladder control (Ballard et al., 2011) may contributes to the death. Risk of

malnutrition cause by the swallowing difficulty and the pneumonia can also cause the death in the AD patients (UK, 2007).

2.7 Risk Factors associated with the AD

2.7.1 Aging

Aging alone is the greatest factor for the development of Alzheimer's disease. People with the age of 65 and above are more likely to have AD compared to younger ones. Risk increases up to 50% in the age of 85 and above ("2010 Alzheimer's Disease Facts and Figures.," 2010).

2.7.2 Genetics

Apolipoprotein E (Apo E) is the gene that code for the protein that is responsible for the transport of cholesterol in the blood, has a close connection with the on-set of AD (Zhao et al., 2014). An isomer of Apo E gene, i.e., Apo E4 targeted as the culprit gene in the disease development. ApoE3 however is considered to be a safeguard against Alzheimer's (Laws et al., 2003).

In case of familial AD chromosomal mutation is considered to be the cause. It occurs in the population younger than 65 age. Around 10% of AD is caused by the mutation on 1, 14, 21 chromosome. Offspring have 50% risk to develop AD if one of the mutated chromosome is inherited (Pichot, 1986) (Weintraub et al., 2012).

2.7.3 Existing Health crises

Health of heart is closely linked with the health of brain. Cardiovascular disease, elevated blood pressure and cholesterol level can increase AD development risk. CVD cause blood vessels damage results in the low blood flow to the brain ultimately causing brain cells death. People having diabetes type 2 are more likely to have Alzheimer disease (Stampfer, 2006).

2.8 Diagnostics

Brain autopsy is the only method to diagnose AD. However physicians conduct combination of mental and behaviour tests along with physical examination in 90% of diagnosis (Zaven S Khachaturian, 1985).

According to the American Psychiatric Associations, statistical manual of mental disorder, Alzheimer is the type of Dementia (Pichot, 1986). Dementia is diagnose by observing the patient who lose the ability to perform intellectual functions, memory loss and other symptoms severe enough to interfere with the daily routine life (Pichot, 1986).

Patient history is an important step in any diagnosis along with the family illness history. General physicians perform multiple tests and examinations to rules out the other possible cause of Dementia i.e., hormonal imbalance and lack of vital vitamins. CT and MRI scans are also done to make sure the symptoms are not because of brain injury, cardiovascular disease, tumours etc. These examinations will also help in observing the brain size and shape also identify senile plaques and NFTs in Alzheimer (Emilien et al., n.d.).

Other cognitive symptoms are identified by performing Mini Mental State Exam that consists of variety of arithmetic tasks, multiple questions been asked to recall new memory, naming things in one go. Patient is then given marks out of 30 with the score above 12 considered to be good and below 12 is considered to be a sign of dementia. This score is gradually decrease in AD patients every year by 2 or more points ("2010 Alzheimer's Disease Facts and Figures.," 2010).

Neurological examinations are performed along with the mental and behaviour tests to assess the neurological functions, muscles strength, glucose level, speech etc. Different techniques for neurological examination are.

- CT
- PET
- MRI

2.9 Treatment

Currently there is no treatment is available for AD. Medication are prescribed only to delay the progression of AD or help in the symptoms in some cases. However exact therapy to reverse the condition is still not present (Carvalho et al., 2015). Medications are prescribed according to the symptoms i.e., cognitive and behavioural (Wimo & Prince, 2010). Memory loss is a cognitive symptom while actions and emotions are behavioural symptoms.

2.9.1 Cognitive symptoms treatment

Cognitive symptoms can be treated by targeting the chemical messenger of the brain. So far two types of medicines i.e., Cholinesterase Inhibitor and Glutamate regulators are approved by FDA to cure these symptoms ("2010 Alzheimer's Disease Facts and Figures.," 2010). Memory and learning are linked with the level of acetyl choline. During the aging this neurotransmitter is slightly decrease causing mild memory problems. However, in Alzheimer this acetyl choline level declines up to 90% causing severe memory loss and behavioural changes. Donepezil,

Galantamine, Rivastigmine are the FDA approved cholinesterase Inhibitors responsible for maintaining nerve cell communications.

Memantine has also been approved by FDA. Memantine functions by regulating the activity of glutamate in brain. Glutamate is also involved with the memory (Sendt et al., 2012). Excitotoxicity is caused by the hyperactivity of glutamate which may cause neurodegeneration (Reisberg et al., 2003). Memantine block NMDA receptor and restrict the excessive binding of glutamate. This can be used for mild to severe AD. Figure 3 summarized the FDA approved drugs along with its mechanism of action and side effects (Carvalho et al., 2015).

Drug	Class and Indication	Mechanism of Action	Common Adverse Effects
Donepezil (FDA-approved in 1996)	Cholinesterase inhibitor prescribed to treat symptoms of mild-to-moderate and moderate-to-severe AD	Prevents the breakdown of acetylcholine in the brain	Nausea, vomiting, diarrhea
Galantamine (FDA-approved in 2001)	Cholinesterase inhibitor prescribed to treat symptoms of mild-to-moderate AD	Prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine in the brain	Nausea, vomiting, diarrhea, loss of appetite, weight loss
Rivastigmine (FDA-approved in 2000)	Cholinesterase inhibitor prescribed to treat symptoms of mild-to-moderate AD	Prevents the breakdown of acetylcholine and butyrylcholine in the brain	Nausea, vomiting, diarrhea, loss of appetite, weight loss, muscle weakness
Memantine (FDA-approved in 2003)	N-methyl-D-aspartate antagonist prescribed to treat symptoms of moderate-t-severe AD	Blocks the toxic effects associated with excess glutamate and regulates glutamate activation	Dizziness, headache, constipation, confusion

Figure 3: This figure shows the list of FDA approved drugs for Alzheimer disease along with the mechanism of action and side effect associated with these drugs.

2.9.2 Behavioural symptoms Treatment

Beside cognitive symptoms AD can also cause anxiety, agitation, delusions along with other behavioural symptoms (Alzheimer & States, n.d.). These symptoms are treated by counselling and rehabilitation along with the medicine being prescribed ("2010 Alzheimer's Disease Facts and Figures.," 2010). Investigating the medicinal side effects that could cause severe damage to the patient psychiatric health, can also be a non-drug treatment. However, for the drug treatment, medicines are prescribed for the specific symptoms like Zoloft is given to the patient if he/she is suffering from depression ("2010 Alzheimer's Disease Facts and Figures.," 2010).

2.10 Economic Burden because of AD

AD comes with a lot of expenses. Annual cost of AD is above 1700 billion USD. In long term care AD patient utilize more health care facility after cancer patient. Medicaid for the low-

income individual is much for higher for the people of 65 and above with AD compared to other elder individual with different disease.

2.10.1 Health Care Usage

Alzheimer patient commonly use more expensive health care services and required more costly care. The table 1 shows the difference between usage and cost of people with AD or dementia with other elder individuals ("2021 Alzheimer's Disease Facts and Figures," 2021)

Table 1: Average consumption of health care facilities by elder people with and without Alzheimer Disease ("2021 Alzheimer's Disease Facts and Figures," 2021)

Healthcare Setting	AD vs. Other Elderly Usage	Average Cost for AD Patient	Average Cost for Other Elderly
Hospital	AD 3 times more visits	$7,663	$2,748
Skilled Nursing Facility	AD 8 times more likely to require service	$3,030	$333
Home Health Care	AD 2 times more likely to require service	$1,256	$282

Materials and Methods

3.1 Software's and Tools used

Different bioinformatic software used in the study are enlisted in the Table 2.

Table 2: List of Software used in Study.

Software	**Application**	**Developer**
Molecular Operating Environment (Version 2015)	structure remodeling and molecular docking	Chemical Computing Group ULC
PyRx	Molecular docking	Source Forge
PyMOL	3-D analysis of ligand – protein complex	Schrödinger, Inc
Ligplot+	2-D representation of ligand protein complex	Roman Laskowski
CLC Drug Discovery workbench 3.0	Atomic insight of chemical compounds	QIAGEN
Cabs flex	Prediction and visualization of protein structure dynamics	Oxford Bioinformatics

3.2 Selection of Ligand and Protein

Tau protein (PDB ID: 2mz7) was selected after literature review. Cabs flex simulation was run against 2mz7 in order to know the suitability of protein for molecular docking, the crystal structure of a microtubule-associated protein human tau (PDB: 2MZ7) (Pradeepkiran & Reddy, 2019) Chain A was used in this study. In order to retrieve the ligand literatures were studied, PubMed, Science Direct, Scopus Google Scholar are used by using searching terms such as "Nigella sativa" or "Black cumin" or "Black seed"(Yimer et al., 2019). Nigella sativa is known for its anti-cancer, anti- viral and multiple therapeutic properties (Aqil et al., 2018).

Four drugs approved by FDA were used for the comparison, so far FDA has only approved five drugs for the treatment of AD, and we have selected four for this study.

3.3 Secondary Structure Prediction:

For the assessment of more structural features of selected protein PDBsum tool was used available at (http://www.ebi.ac.uk/pdbsum) PDBsum give in depth information structural features of peptides, proteins and their ligands (Laskowski et al., 2018). Enlarged secondary structure is given in Figure 4.

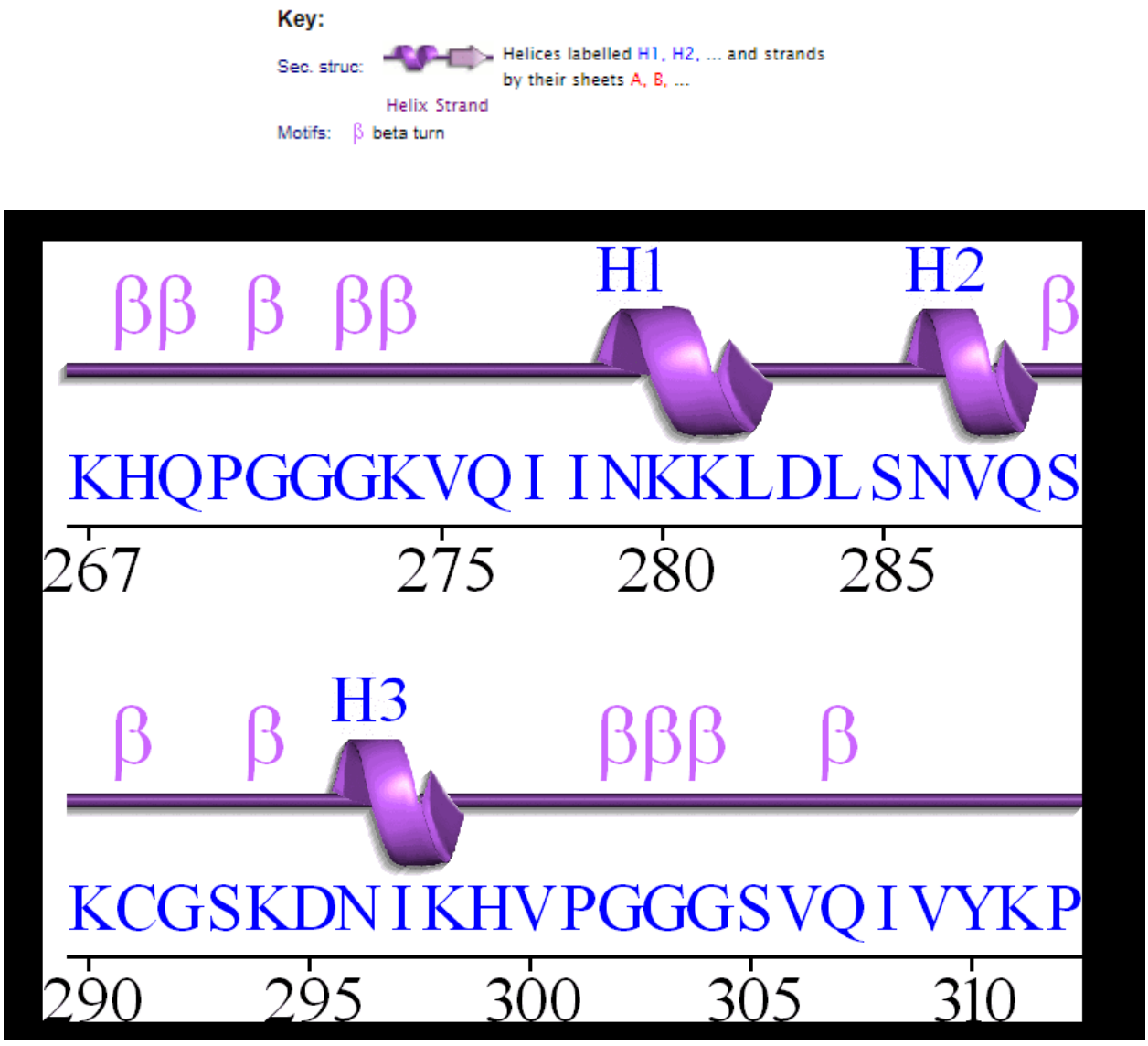


Figure 4: Secondary structure of human Tau protein 2mz7: Secondary structure assessment reflects three Helices.

3.4 Protein Structure flexibility and Structure Profile:

CAB-Flex is an online open-source tool to predict the protein structural fluctuation(Jamroz et al., 2014). Target protein 2mz7 was submitted to the server and gives out the fluctuation profile of protein. Figure 5 represents the RMSF of the 2mz7 protein which shows that the protein is overall stable and can be used for molecular simulation.

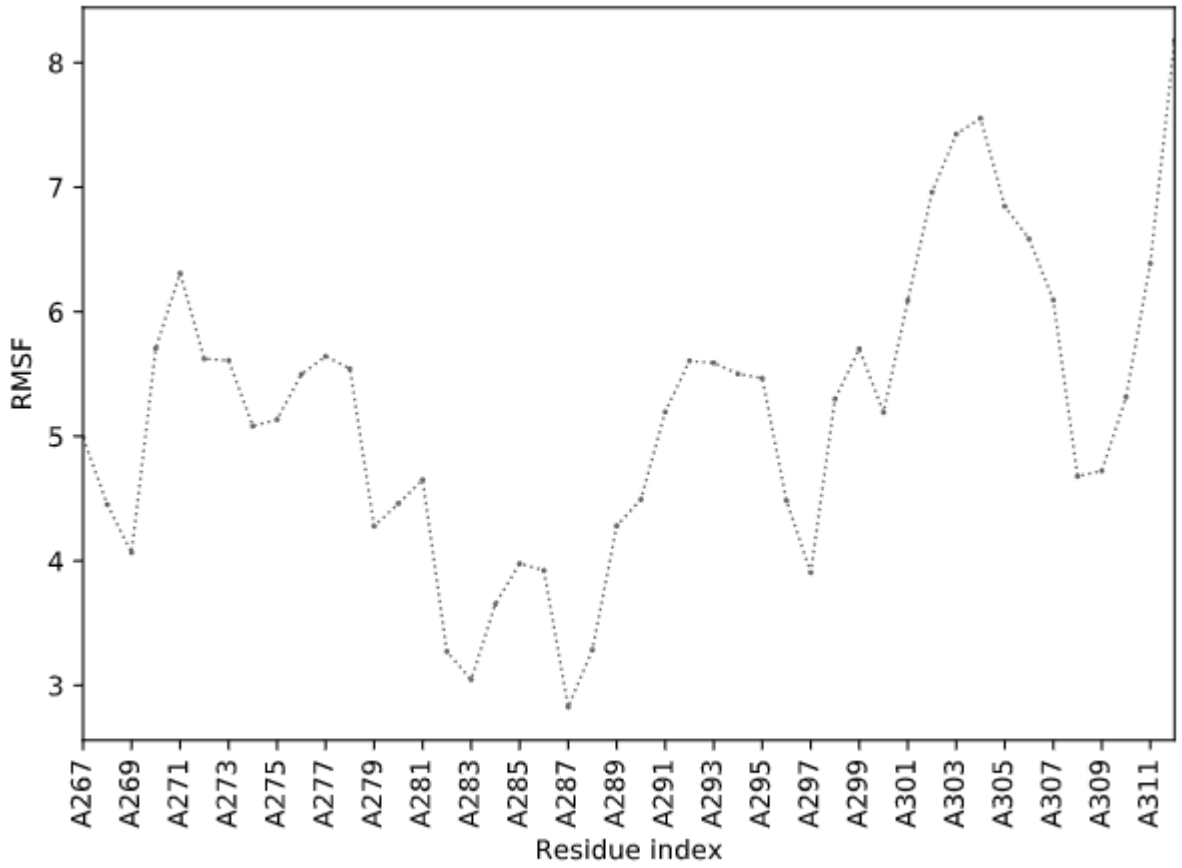


Figure 5: Root mean square fluctuation during cab flex analysis shows the fluctuation, but the overall structure is stable.

3.5 Docking through Molecular Operating Environment

Molecular operating environment is a platform use for drug discovery that offers several tools like modelling, simulation, molecular docking, and visualization all in one package (Merz Jr, Kenneth M and Ringe, Dagmar and Reynolds, 2010).

3.5.1 Ligand and protein preparation: 3- Dimensional structure of human Tau protein was downloaded from Protein data Bank under PDB ID: 2mz7 (Pradeepkiran & Reddy, 2019) 2-Dimensional structure of 2mz7 is given in figure 7 and properties of 2mz7 is reported in table 3**.** Chemical compounds from Nigella sativa were taken from PUBCHEM in .sdf format (Bouchentouf & Missoum, 2020) Lipinski's physicochemical parameters rule (Lipinski et al., 2001) were also studied for each ligand and calculated after energy minimization module implanted in MOE by two different platforms i.e. CLC Drug Discovery workbench and open access tool available online (Lipinski Rule of Five (scfbio-iitd.res.in)) and reported in table 4. Chemical structures and properties of FDA approved drugs for Alzheimer disease(*FDA-Approved t Reatments for Alzheimer ' s*, n.d.) are reported in Table 5.

3.5.2 Energy Minimization: Energy minimization module implanted in MOE was used for energy minimization of 2mz7, water molecules were absent in this structure, so the docking site was exposed. Using Hamiltonian AM1(Austin model 1) and MMFF94x (Merck molecular force field 94x) energy of ligand i.e., both drugs and natural compounds from Nigella sativa L were minimized (Bouchentouf & Missoum, 2020). Active site that will interact with the ligand (Soga et al., 2007) active site of 2mz7 was identified by the site finder tool in MOE shown in Figure 6.

3.5.3 Docking: Docking module present in MOE was used that consist of positioning and placement of ligands. Default tools of MOE under default conditions were used for the prediction of ligand and binding sites of protein interaction (Karthikeyan & Vyas, 2014). Two different placement methods i.e., Triangle Matcher and Alpha PMI among different placement options were followed and only the result with best score are presented.

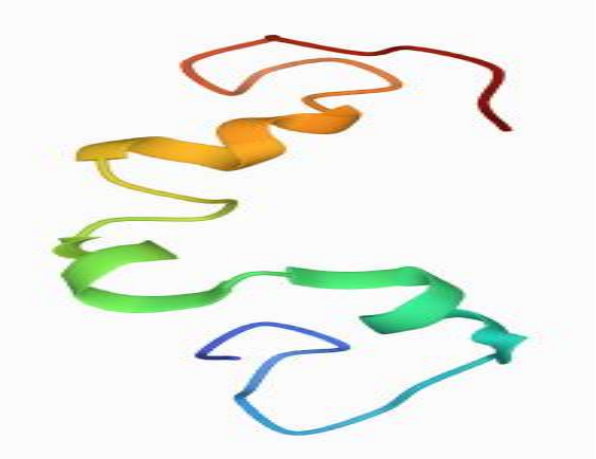


Figure 6: Structural representation of human tau protein chain A (PDB ID 2mz7)

Table 3: list of chemical properties of 2mz7 (Kadavath et al., 2015)

Protein	Structure of Tau (267-312) bound to Microtubules
PDB ID	2mz7
Classification	Protein binding
Organism	Homo Sapien
Total structural weight	4.89 kda
Method	Solution NMR
Sequence length	46
Chain	A

Table 4: Lipinski's physicochemical parameters of selected compounds from Nigella *Sativa* obtained from clc drug discovery work bench.

Ligand	**Name**	**PubChem ID**	**Lipinski's parameters**	
1	Nigellicine	11402337	**Properties**	**Value**
			Mass	233.000000
			Hydrogen bond donor	1
			Hydrogen bond acceptor	3
			LOGP	-0.443830
			Molar Refractivity	57.564800
2	Nigellidine	136828302	**Properties**	**Value**
			Mass	294
			Hydrogen bond donor	1
			Hydrogen bond acceptor	2
			LOGP	1.825940
			Molar Refractivity	82.504288
3	Nigellimine	20725	**Properties**	**Value**
			Mass	203
			Hydrogen bond donor	0
			Hydrogen bond acceptors	3
			LOGP	2.145230
			Molar Refractivity	52.893997

4	Carvacrol	10364	**Properties**	**Value**
			Mass	150
			Hydrogen bond donor	1
			Hydrogen bond acceptors	1
			LOGP	2.161600
			Molar Refractivity	48.491791
5	Thymol	6989	**Properties**	**Value**
			Mass	150
			Hydrogen bond donor	1
			Hydrogen bond acceptor	1
			LOGP	2.161600
			Molar Refractivity	48.491791
6	Thymoquinone	10281	**Properties**	**Value**
			Mass	164
			Hydrogen bond donor	0
			Hydrogen bond acceptor	2
			LOGP	1.977800
			Molar Refractivity	46.871994
7	Dithymoquinone	398941	**Properties**	**Value**
			Mass	328
			Hydrogen bond donor	0

			Hydrogen bond acceptors	4
			LOGP	3.913599
			Molar Refractivity	93.963989
8	Thymohydroquinone	95779	**Properties**	**Value**
			Mass	166
			Hydrogen bond donor	2
			Hydrogen bond acceptors	2
			LOGP	1.861800
			Molar Refractivity	49.370590

Table 5: Lipinski's physicochemical parameters of the FDA approved drugs

Ligands	**Mass (Dalton)**	**Hydrogen Bond Donor**	**Hydrogen Bond Acceptor**	**LOGP (lipophilicity)**	**Molar Refractivity**
Donepezil	233	1	3	0.443830	57.564800
Galantamine	294	1	2	1.825940	82.504288
Rivastigmine	203	0	3	2.145230	2.145230
Memantine	150	1	1	2.161600	2.161600

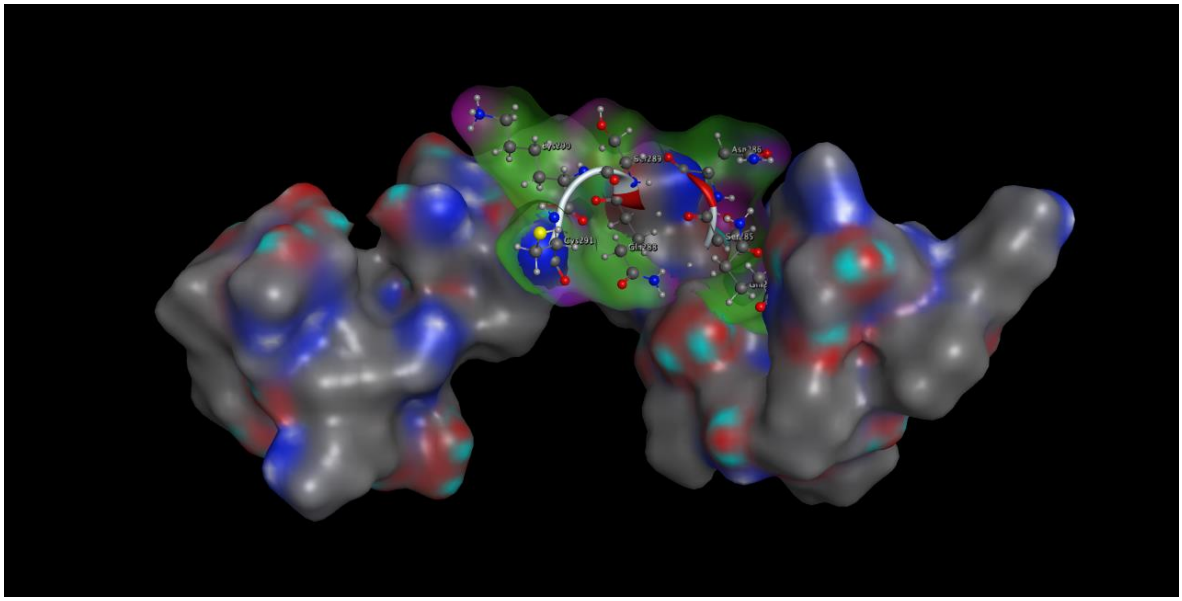


Figure 7: This figure is obtained from the molecular operating environment and shows the 3-Dimenssional structure of 2mz7. The ball and stick models in the green region show the active residue of tau while the solid structure represents the non-active residues

3.6 Molecular Docking through PyRx

3.6.1 PyRx: Pyrx is an open-source computer aided drug discovery software. This is used for the similar purpose as for MOE. Results obtained from Pyrx are compared with the results obtained from MOE.

3.6.2 Docking: For docking through PyRx protocol propose by Sargis Dallakyan and Arthur J. Olson was followed (Dallakyan, Sargis; Olson, 2015). Ligand and protein format was same as MOE i.e., .sdf and .pdb, respectively. First receptor molecule was converted into PDBQT form. Energy of ligand molecules using field strength in the MMFF94 (Merck molecular force field) (Tosco et al., 2014) was minimized by tool in Open Babel software package implanted in PyRx (O'Boyle et al., 2011) and convert all the molecules in PDBQT format step wise. Vina wizard module in PyRx was used for docking and the results obtain for both drugs and natural compounds are presented in table.

3.6.3 Ligand and receptor interaction

In order to analyse the interaction between ligand and receptor, the docked molecules were submitted to pyMOL (Siam et al., 2017) to produce a ligand – receptor complex. pyMOL is an open-source tool use for Molecular Visualization. Ligand-receptor complex was then analyse

through bioinformatic tool Ligplot+ that is used for 2-D representation of the Protein-Ligand Complex (Laskowski & Swindells, 2011).

RESULTS

Human Tau protein under PDB ID:2mz7 was selected as a target protein and natural compounds from Nigella Sativa are used as candidate ligand and synthetic compounds are the drugs approved by FDA. Two different tools, Molecular Operating Environment and PyRx are used for the molecular docking, PyMOL was used for ligand-protein complexes visualization and Ligplot for the analysis of the ligand-protein interaction.

4.1 Molecular operating environment simulation analysis

For the evaluation of protein-ligand interaction docking program in MOE. All the docking was carried out between the human Tau protein and the synthetic and natural compounds with the range of default docking parameters. Binding energy obtained from the MOE Tool are presented in the table six. Left table shows the energy of natural compounds and synthetic compounds are present at the right.

Table 6: Docking score of 2mz7 with Nigella Sativa Compounds and selected drugs by using MOE

Natural Compounds	Score (Kcal/mol)
Nigellicine	-4.0788
Nigellimine	-4.9886
Carvacrol	-4.4434
Nigellidine	-4.9866
Thymol	-3.9265
Thymoquinone	-4.2207
Dithymoquinone	-5.04495
Thymohydroquinone	-4.0834

Synthetic Compounds	Score (Kcal/mol)
Donepezil	-5.6872
Galantamine	-5.4644
Memantine	-3.9462
Rivastigmine	-4.5165

Dithymoquinone gives lowest binding energy (**-5.04495 Kcal/mol**) when binds with the 2mz7 as compared to the other compounds docked with MOE. This is nearest to the score obtained by Galantamine and 2mz7 complex and better compared to the Memantine and Rivastigmine (i.e., **-3.9462 Kcal/mol** and **-4.5165 Kcal/mol** respectively). Docking complex of Dithymoquinone and 2mz7 is shown in figure 8 and figure 9 shows the interaction of compounds with the amino acids. Table 5 and 6 reports the rest of Nigella *sativa* compounds and the drugs interactions with amino acids.

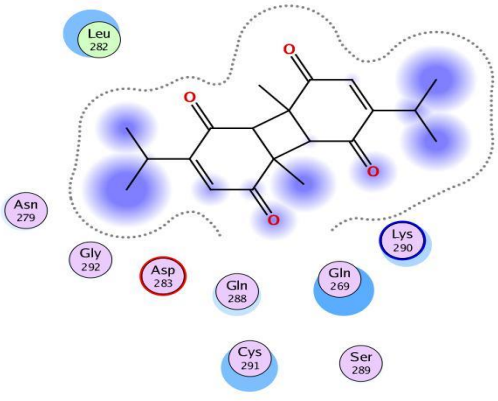


Figure 8: Two-dimensional interaction of **dithymoquinone** and **2mz7**

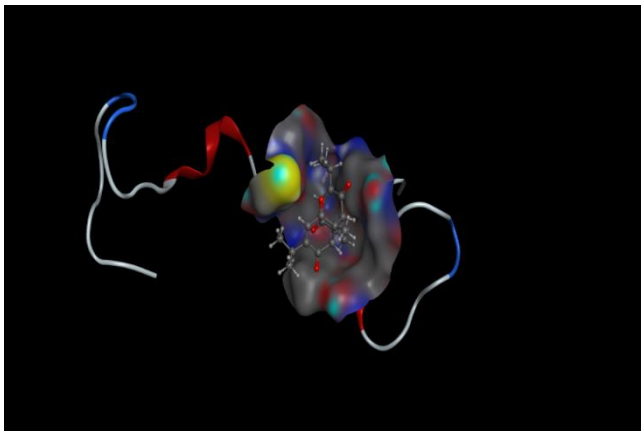
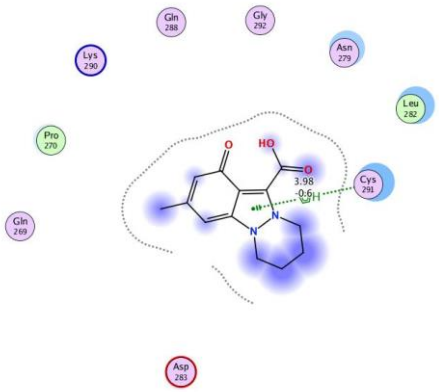
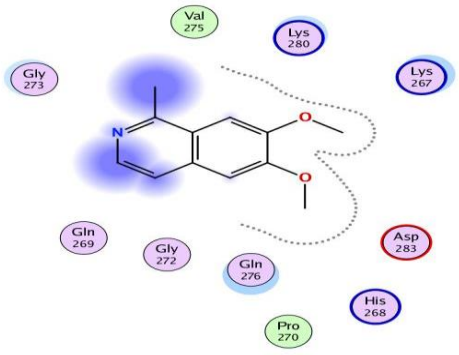
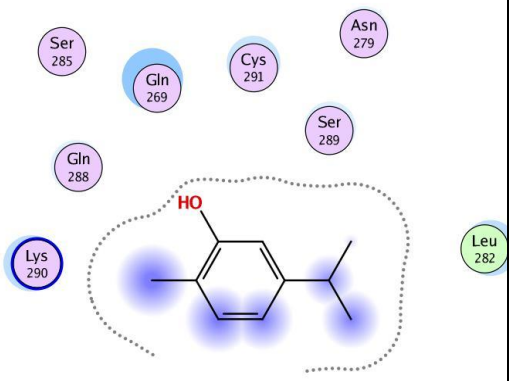
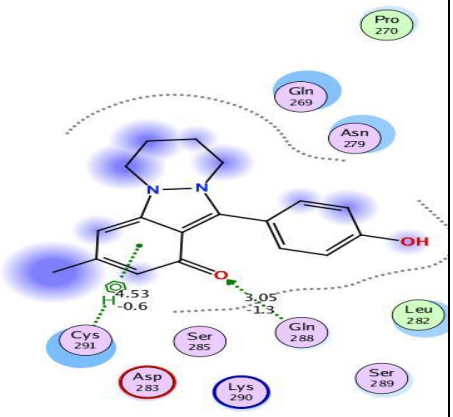
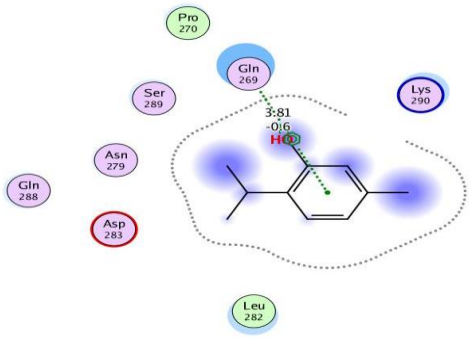


Figure 9: Three-dimensional diagram shows the interaction of d**ithymoquinone** and **2mz7** protein. Solid surface shows the interacting site and the ball and stick structure represent the

Active residues of 2mz7 that interacts with ligands using MOE are **(GLN269 SER285 ASN286 GLN288 SER289 LYS290 CYS291).** Detailed analysis of the ligands and protein interaction are presented in the table 7, where it is describe how the and which amino acid residue is in the contact with the ligand , bonding type , energy and bonding distance are also explained.

Table 7: detail analysis of natural compounds interaction with 2mz7 obtained from Molecular operating environment demonstrating the type of interaction, bond length and binding energy

Ligand	**Structure interactions**	**Type of interactions**
Nigellicine		Hydrogen bond (π – H) with amino acid Cys 291 is possible with almost the energy of -0.6 Kcal/mol and distance of 3.98Å
Nigellimine		Except wander walls no visible interactions exist, blue region shows the ligand exposure

Carvacrol		Except wander walls no visible interactions exist
Nigellidine		Two type of Hydrogen interaction are possible, Cys 291 form bond (π – H) with almost the distance of 4.53 Å and energy of -0.6 Kcal/mol while Gln 288 act as a Hydrogen acceptor
Thymol		Hydrogen bond (π – H) with amino acid Gln269 is possible almost with the energy of -0.6 Kcal/mol and distance of 3.81Å

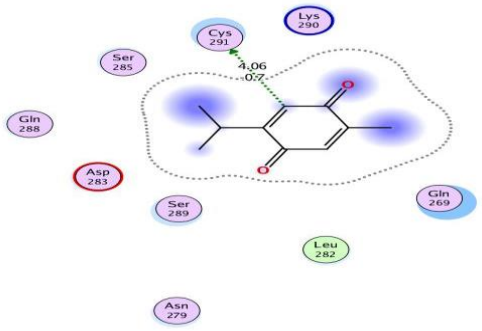
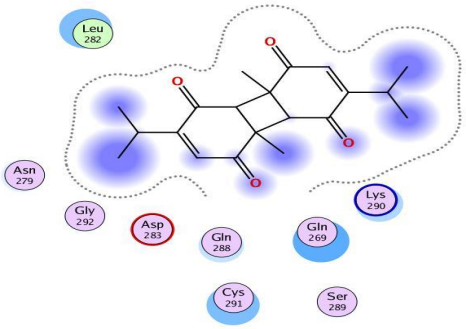
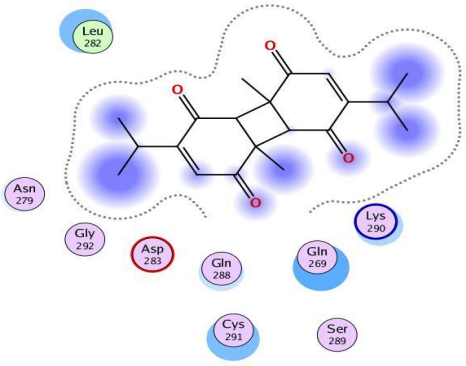
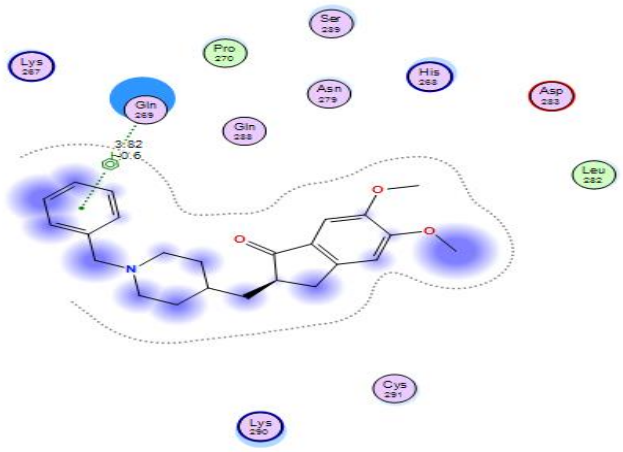
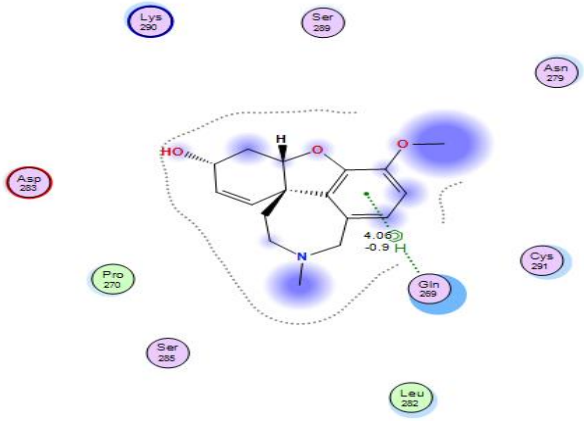
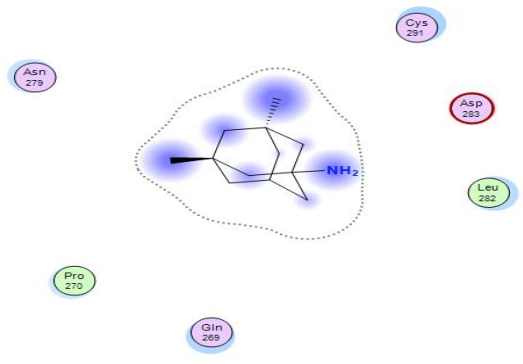
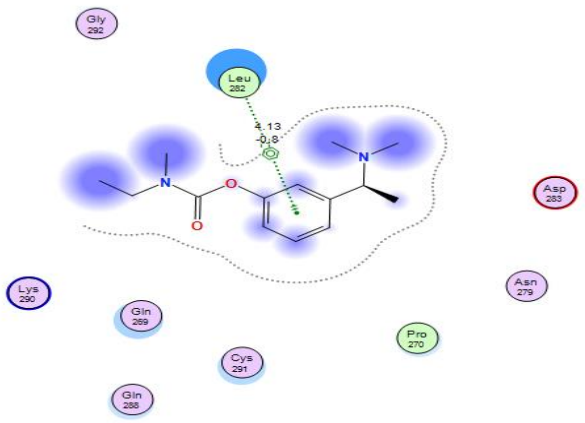
Thymoquinone		Only one type of Hydrogen interaction exists, Cys 291 act as a Hydrogen acceptor with almost the distance of .06 Å and energy of -0.7 Kcal/mol
Dithymoquinone		Except wander walls no visible interactions exist
Thymohydroquinone		Except wander walls no visible interactions exist

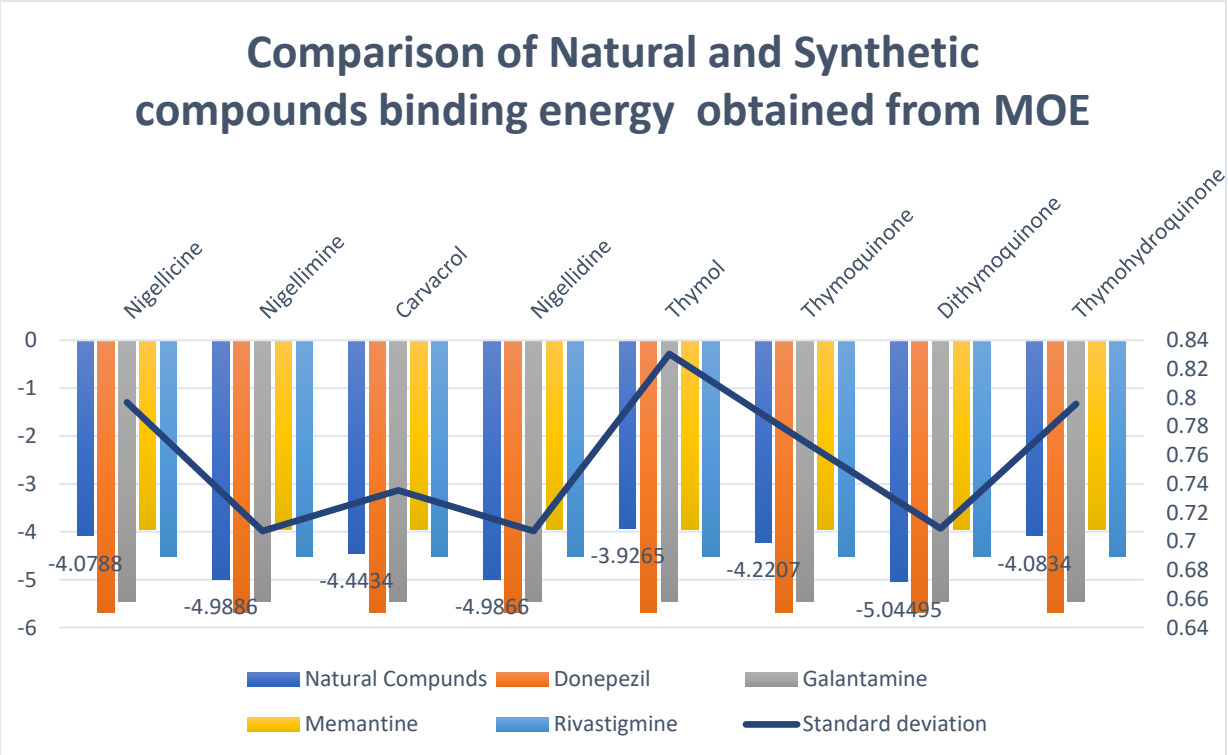
Table 8: Detail analysis of 2mz7 interaction with synthetic drugs using Molecular operating environment demonstrating the amino acid interaction with the ligand compounds and type of interaction.

Ligand	**Structure interactions**	**Type of interactions**
Donepezil		Only one type of Hydrogen interaction exists, Gln 269 act as a Hydrogen acceptor with almost the distance of 3.82 Å and energy of -0.7 Kcal/mol
Galantamine		Only one type of Hydrogen interaction exists, Gln 269 act as a Hydrogen acceptor with almost the distance of 4.06 Å and energy of -0.9 Kcal/mol
Memantine		Except wander walls no visible interactions exist

Rivastigmine		Only one type of Hydrogen interaction exists, Leu 282 act as a Hydrogen acceptor with almost the distance of 4.13 Å and energy of -0.8 Kcal/mol

4.2 Binding Energy Evaluation:

Binding energy obtained from MOE are evaluated, standard deviated of each natural compound from the synthetic compounds were calculated and presented in combine chart.



Graph 1: The combine graph shows the comparison among natural and synthetic compounds. Values at the left side represent the binding energy while the right side show the standard deviation.

4.3 PyRx vina analysis

PyRx tool was also used for the evaluation of Nigella Sativa compounds and to compare their binding affinity to the selected drugs. Table 9 represents the binding energy of the natural and synthetic compounds with 2mz7.

Table 9: Docking score of 2mz7 with Nigella *Sativa* Compounds and selected drugs by using PyRx

Natural Compounds	**Score (Kcal/mol)**
Nigellicine	-5.3
Nigellimine	-4.6
Carvacrol	-4.4
Nigellidine	-5.8
Thymol	-4.3
Thymoquinone	-4.4
Dithymoquinone	-6.1
Thymohydroquinone	-4.3

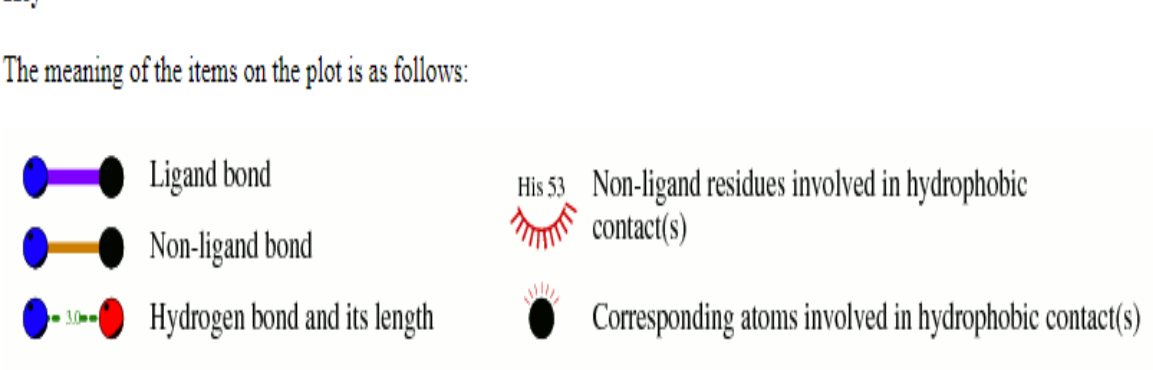
Synthetic Compounds	**Score (Kcal/mol)**
Donepezil	-5.7
Galantamine	-6
Memantine	-4.4
Rivastigmine	-4.8

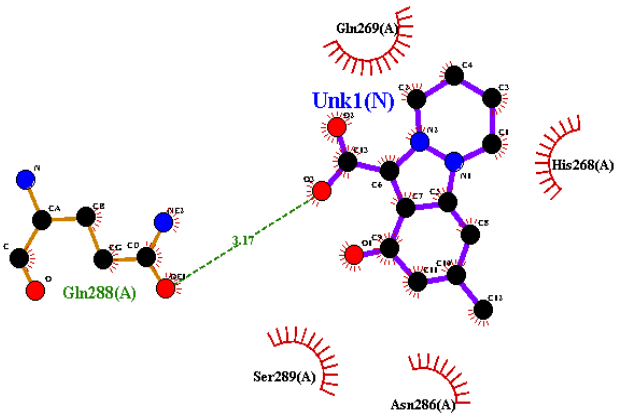
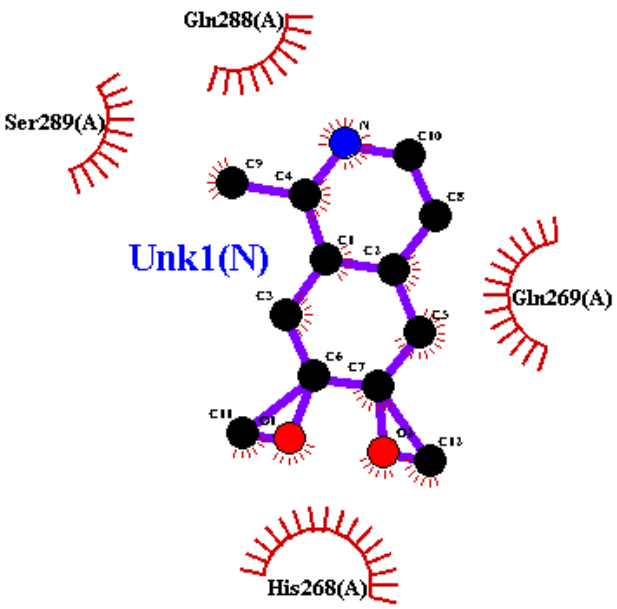
In comparison to MOE, results obtained by using PyRx vina shows that dithymoquinone (PubChem ID: 39894) gives the best result (**-6.1 Kcal/mol**) which is better than the synthetic compounds. Nigellidine give the binding energy (**-5.8 Kcal**/mol) which is nearest to Galantamine (**-6 Kcal/mol**). Nigellidine gives better binding energy than Donepezil (**-5.7 Kcal/mol**), Memantine (**-4.4 Kcal/mol**) and Rivastigmine (**-4.8 Kcal/mol**). Ligplot analysis of dithymoquinone and 2mz7 shows that **Gln 269** and **Asn 286** make a Hydrogen bond with the ligand with the distance of **2.80Å** and **3.22Å**, respectively. Other amino acids show hydrophobic interaction with ligand.

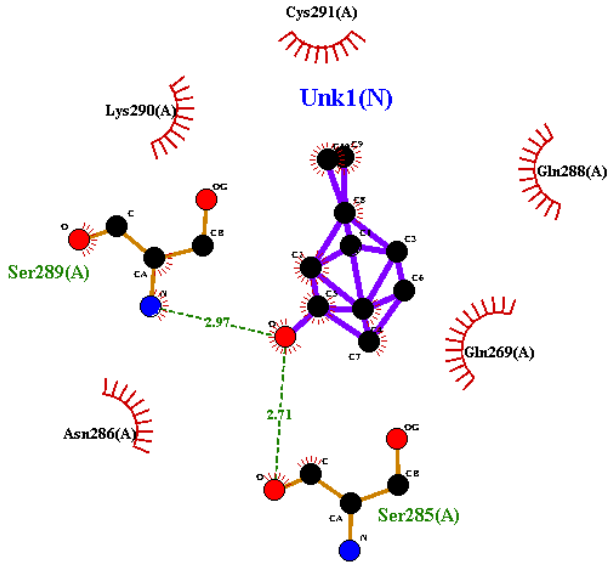
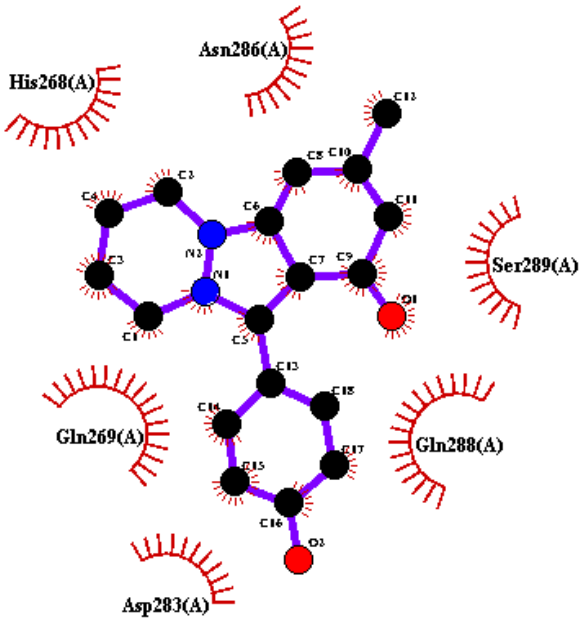
4.4 Ligplot analysis

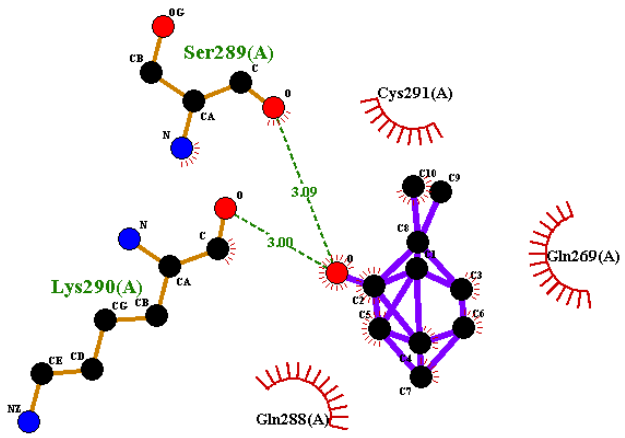
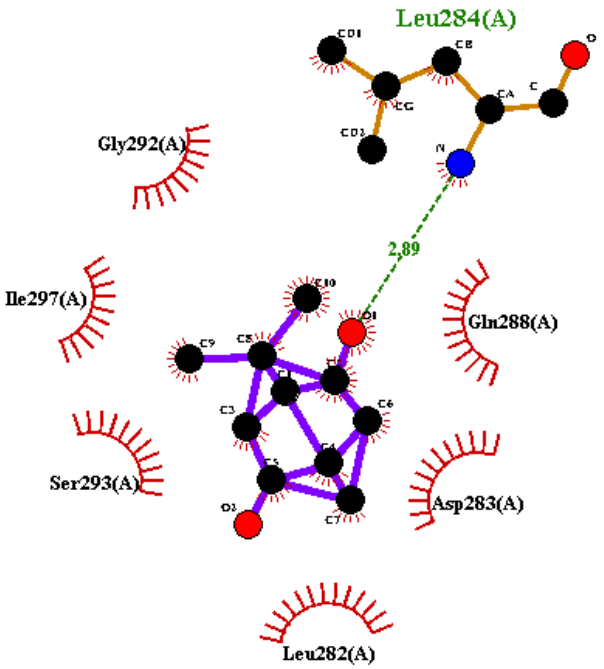
Ligplot is used for the evaluation of the protein-ligand complex interaction. PDB files retrieve from PyMOL were analyse by using Ligplot. 2-D representation of protein-ligand complexes are presented in table 9 which shows the bonding types between the amino acids and ligands and their binding affinity. In Nigellicine, Gln 288 makes a Hydrogen bond with the distance of 3.17Å while in Carvacrol, Ser 289 and Ser 285 make a Hydrogen bond with the ligand with the distance of 2.97Å and 2.71Å, respectively. Other amino acids show hydrophobic interaction with ligand molecule. In case of Thymol, Ser 289 and Lys 290 make a Hydrogen bond with the ligand with the distance of 3.09Å and 3.00Å, respectively. Other amino acids show hydrophobic interaction with ligand. Leu 284 makes a Hydrogen bond with the thymoquinone with the distance of 2.89Å and Leu 282 makes a Hydrogen bond with the Thymohydroquinone with the distance of 3.34Å while other amino acids show hydrophobic interaction only. No Hydrogen bonding is present in Nigellidine and Nigellimine only hydrophobic interaction exists. To compare the interaction, type synthetic compounds in complex with 2mz7 are presented in table 10 with detailed analysis.

Table 9: 2-D representation of natural compounds interaction with 2mz7 using Ligplot



Natural Compounds	Structure interactions
Nigellicine	
Nigellimine	

Carvacrol	
Nigellidine	

Thymol	
Thymoquinone	

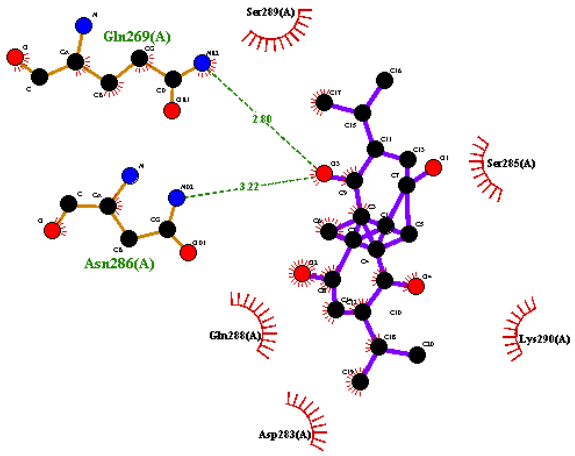
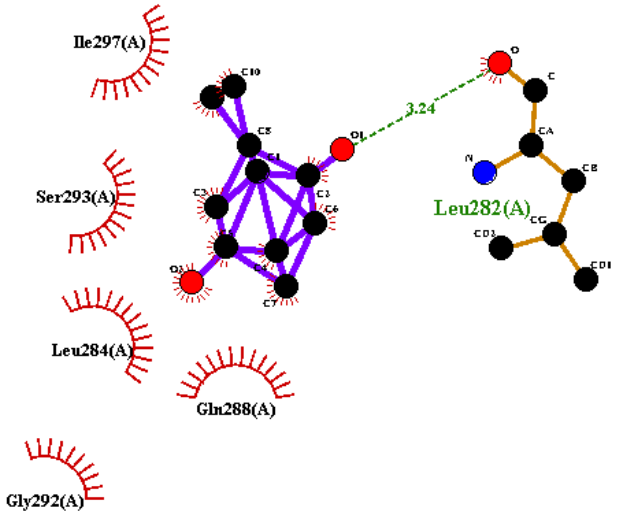
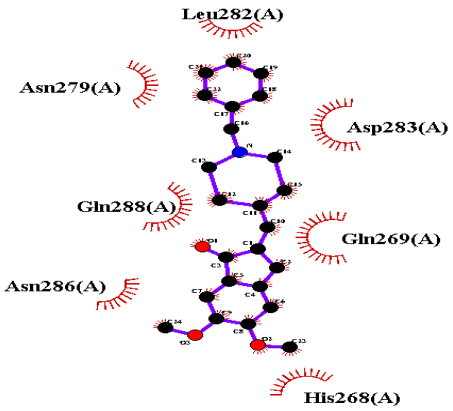
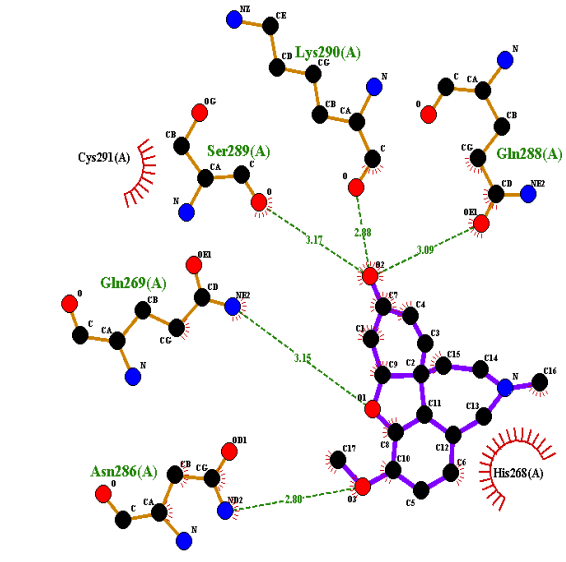
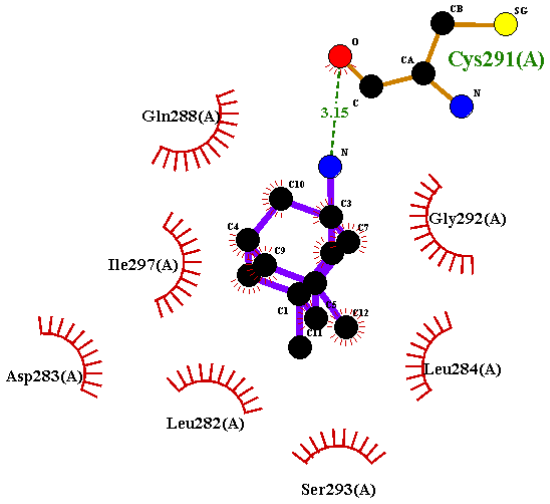
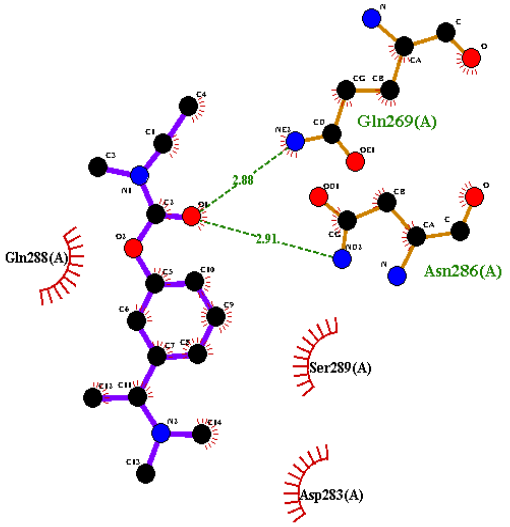
Dithymoquinone	
Thymohydroquinone	

Table 10: detail interpretation of synthetic compounds interaction with 2mz7 using Ligplot. Hydrogen bonds among ligand and protein residues were represented by dotted green colour lines. While residues containing hydrophobic interactions, were shown as circular red lines

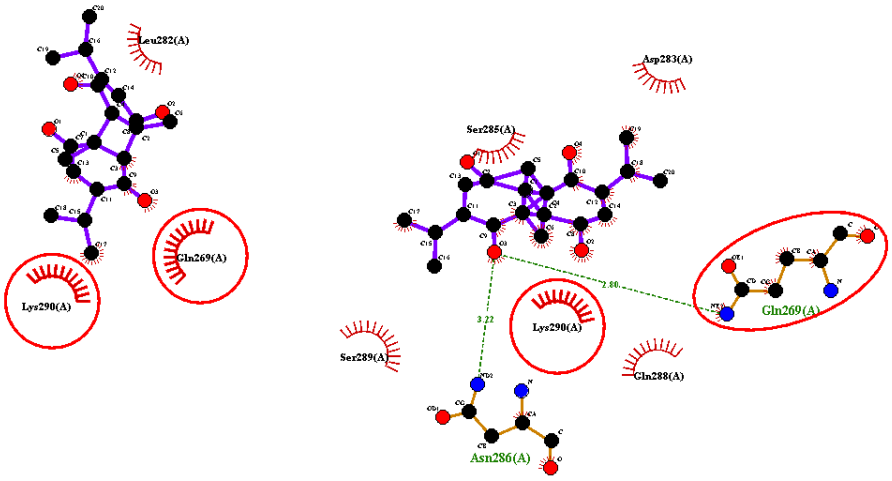
Synthetic Compounds	**Structure interactions**	**Type of interactions**
Donepezil		Except hydrophobic interaction walls no visible interactions exist
Galantamine		Five types of Hydrogen interaction exist, Gln 288, Lys 290, Ser 289, Gln 269, Asn 286 act as a Hydrogen acceptor with almost the distance of 3.09, 2.88, 3.17, 3.15, 2.80 Å respectively while His 268 shows hydrophobic interaction

Memantine		Only one type of Hydrogen interaction exists, Cys 291 act as a Hydrogen acceptor with almost the distance of 3.15 Å while the other amino acids show hydrophobic interaction only
Rivastigmine		Two types of Hydrogen interaction exist with Gln 269 and Asn 286 the distance of 2.88 and 2.91 Å respectively while the other amino acids show hydrophobic interaction

4.5 Comparison of docking complexes

Two different tools were used to identify the potent inhibitor of tau hyperphosphorylation among the Nigella *sativa* derivatives. Dithymoquinone standout in both tools i.e., MOE and PyRx, respectively. Side wise comparison of the outcome of both tools were carried out in Ligplot presented in table 12. Pretty much resemblance can be seen in both results. Red circles around the amino acids show the residues that are common in both interactions except for Nigellimine which show no similarity in between the results obtained from MOE and PyRx.

Table 12: Sidewise comparison of Dithymoquinone interaction with 2mz7 obtained from MOE and PyRx shows the common interacting amino acid residues of 2mz7 in the red circles.

Protein ligand Complex	MOE and PyRx
Dithymoquinone	

DISCUSSION

During the past decade, computational approaches have shown their success and power in assisting drug discovery and development. Keeping the increasing number of Alzheimer disease patients, searching for potential inhibitors to combat the disease in efficient time, and in a cost-effective manner is significantly important. Taking advantage of all available information on tau this study investigates the potential inhibitors of Alzheimer disease. The derivates of the natural compound, Nigella *sativa* were used to target the tau phosphorylation sites. A combination of computational tools; including molecular docking, virtual screening, molecular dynamics simulations, and Ligplot analysis, were used to identify a potent inhibitor for Alzheimer disease treatment. The insights into the tau inhibitors and their complexes, as well as their binding energies, were explained. Our findings provide a foundation which would be helpful for further research.

FDA approved last drug for the treatment of Alzheimer's disease 17 years ago as the time passes number of AD patients increases following the struggles of medical technology development. Public health is a fundamental criterion for the development of a stable nation free of diseases. It also successively decreases personal healthcare costs. AD is the cause of poor lifestyle among the elderly population mostly. Both effective drugs and early detection of disease is necessary to cope up with this type of Dementia. However only experimental techniques could not help the quicker approach for the drug production. Black seed compounds, specifically Dithymoquinone, a bioactive isolate of Nigella Sativa and isomer of thymoquinone seems to have potential to bind with Tau protein and may prevent the formation of neuro fibrillary tangles.

With the goal of identify the potent inhibitor for Alzheimer disease this study utilizes the available crystal structure or tau protein (PDB id 2mz7) as a starting point. Initially, reproducing the experimental data indicated a successful and robust docking protocol with molecular operating environment and PyRx. In this study Nigella *sativa* was taken as a drug discovery candidate. Lipinski rule of five was applied to determine the drug likeness of the derivatives of the Nigella *sativa* by evaluating the molecular mass, hydrogen bond acceptance and donor and the lipophilicity. The results obtained from CLC drug discovery work bench reveals that all the derivatives of Nigella *sativa* are pharmacologically active for oral administration.

Molecular study examined the interaction of Nigella *sativa* derivatives (Nigellidine, Nigellicine, Nigellimine, Carvacrol, Thymol, Thymoquinone, Dithymoquinone, Thymohydroquinone) with target receptor tau protein 2mz7. Docking complex images for all these derivatives containing interaction and surface maps are demonstrated. The docking results suggested that Dithymoquinone with -5.04 kcal/mol from Molecular operating environment and -6.1 Kcal/mol from Pyrx has the best energy score and potent interacting inhibitor as compared to the other derivatives. Whereas Thymol, has shown the lowest energy score of -3.92 Kcal/mol. Molecular docking results suggested variation from the best interaction to moderate binding interaction, which were based upon following observations: a) The energy scores (S) represent the range of best binding interactions to moderate ones which range from -5.0492 to -4.98.

b) The ligation mode was mostly covering N, and benzene ring sites.

c) The main binding active amino acid residues of receptor were leucine, Glutamine, and serine respectively.

d) The hydrogen bond interactions include almost all types of bonds, such as H-donor, H acceptor, and π-H except from π-π.

Ligplot analysis was also employed to re analyse the ligand-protein complex interactions. Above discussion concludes that Dithymoquinone is the best inhibitor as compared to the synthetic drugs and the study also exhibited interacting images of docking complexes explaining the overall docking results. All these findings collectively indicate targeting tau protein for the inhibition of Alzheimer disease will gives better results unlike the Amyloid targeted inhibitors. Our research demonstrated Nigella *sativa* as a potential inhibitor of Alzheimer's disease with the help of interaction analysis and bioinformatic study. Aside from interaction analysis, bioinformatic tools were employed to study protein-ligand complex interactions. The predicted protein-ligand contacts through LIGPLOT study shows the amino acid residues involve in the phosphorylation of tau are present in the amino acid residues interacting with the ligands.

Conclusion and Future Prospects

Conclusions

In this study, specific interaction between the human tau protein and the compounds from Nigella *Sativa* were investigated and compare the already present drugs approved by FDA for AD through bioinformatics approach using molecular simulation and visualization tools like MOE, PyRx, PyMOL, Ligplot. We evaluate all the natural compounds from the Nigella *Sativa* in two different tools and Dithymoquinone standout among all-natural compounds and sidewise comparison reveals that Dithymoquinone has the lowest binding energy among the natural compounds i.e., **-5.04 Kcal/mol** and **-6.1kcal/mol** from MOE and PyRx, respectively and both tools reveal the **Gln 269**, and **Lys 290** are the common amino acid residues that interacts with dithymoquinone.

Future prospects

The purpose of the study was to use immunoinformatic approaches to predict the potential compound of Nigella *sativa* that can be used for Alzheimer's treatment. e. In silico analysis reveals that dithymoquinone, the isomer of thymoquinone could interact with tau protein better than already present drugs approved by FDA for AD. These results indicate Nigella *sativa,* and its derivatives could possibly be approved as novel therapeutic approach against Alzheimer's disease.

Chapter 6

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