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RESEARCH ARTICLE

Molecular Docking Studies of Plant Derived Natural Products for the Treatment of Neurological Disorders

Jauhari Nupur, Saxena Supriya, Gupta Sakshi, Bharadvaja Navneeta*

Department of Biotechnology, Delhi Technological University (formerly Delhi College of Engineering), New Delhi, Delhi, India.

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ABSTRACT

Neurological disorders like Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Schizophrenia and Major Depressive Disorder pose a major threat to lives of human beings and do not have a definite cure yet. Computational methods like Molecular Docking can help in the design of structure-based rational drugs for these types of disorders. Selected plant-derived compounds like Triterpenoids, Flavonoids, Capsaicinoids and other lead compounds that exhibit the potential to inhibit different enzymes or receptors and act upon targets in cellular pathways were subjected to molecular docking using HEX 8.0. Numerous enzymes and receptor proteins involved in cellular pathways of protein aggregation and degeneration of motor neurons like COX-2, NF-κB, Acetyl cholinesterase and D2-Dopamine receptor were docked. The main objective of the paper is to calculate interaction energies between natural compounds and these receptors was measured and compared using Hex 8.0 through molecular docking. This *in-silico* method adopted in the present study would thus help in identifying novel compounds for treatment of neurological disorders and subsequently development of drugs.

KEYWORDS

Molecular Docking, Computational Methods, Neurological Disorders, Alzheimer's Disease, Parkinson's Disease, Schizophrenia, Drug Development

INTRODUCTION

Neurodegenerative diseases like Alzheimer's, Parkinson's, Amyotrophic lateral sclerosis (ALS) and Schizophrenia have similar pathogenesis mechanism involving accumulation and deposition of wrongly folded proteins, which leads to progressive central nervous system diseases[1]. Alzheimer's disease is a chronic neurological disorder associated with degeneration of neurons in the hippocampus and cortical region, leading to cognitive and memory impairments.

***Address for Correspondence:**
Dr. Navneeta Bharadvaja,
Delhi Technological University,
(Formerly Delhi College of Engineering),
Bawana RoadDelhi-110042, India.
E-Mail Id: navneetab@dtu.co.in

Parkinson's disease is characterised by progressive dopamine-generating neurons loss in the Substantianigra. Amyotrophic lateral sclerosis leads to muscle weakness and atrophy due to degeneration of upper and lower motor neurons. Schizophrenia is a mental disorder associated with abnormal social behavior where the patient has difficulties in recognizing what is real. Major Depressive Disorder is caused by increased levels of receptors like serotonin and norepinephrine, and leads to low esteem and mood swings among patients. Capsaicin, Nordihydrocapsiate (derived from *Capsicum Anuum*), Bacoside-A (derived from *Bacopa Monnieri*) and Avicins (derived from *Acacia victoriae*) are found to have anti-inflammatory activities and inhibit NF- kB activation[2,3,4].

Swiss-Prot target prediction suggests possibilities for interactions of Capsaicin and related compounds and Avicins with COX-2 as well. Inhibition of NF-Kβ helps in the treatment of neurological disorders like Parkinson's disease[3]. COX-2 inhibition protects the degeneration of motor neurons and has been shown to increase mouse model longevity in case of Amyotrophic Lateral Sclerosis[5]. Lead compounds like Lobeline (derived from *Lobelia inflata*) and Lupanine (derived from *Lupinus polyphyllus*) have the potential to inhibit Dopamine D2 receptor[6]. Dopamine D2 receptor antagonism helps in the treatment of Alzheimer's disease by suppressing neuro-toxicity and Tau aggregation[7]. In addition, inhibition of Dopamine D2 Receptor has shown improvements in symptoms of patients suffering from Schizophrenia[8]. Natural Phenolic compounds like Curcumin (belonging to ginger family *Zingiberaceae*) and Naringenin (derived from grapefruits and tomatoes) have antioxidant abilities and the potential for neuroprotection[9]. Swiss-Prot target prediction for Saligcinnamide (a steroidal alkaloid derived from *Sarcococca saligna*[10] show possibilities for binding interactions with Acetylcholinesterase and Dopamine D2 Receptor. Acetyl cholinesterase is involved in the hydrolysis of the neurotransmitter acetylcholine. Inhibition of this enzyme plays an important role in treatment of Alzheimer's disease[11]. Nitidine (derived from *Zanthoxylum)* has the potential to inhibit Catechol-o-methyl transferase and Acetylcholine stearase. Catechol-o-methyl transferase metabolizes Levadopa and makes it unavailable for conversion to Dopamine. Inhibition of COMT can thus help in improving effects of Parkinson's disease. Macluraxanthone C (derived from *Maclura pomifera*) has anti-inflammatory properties and Swiss-Prot target prediction suggests interactions with Monoamine oxidase A and Cox-2. Flavonoids like Apigenin (derived from parsley), Rutin and Quercetin (derived mainly from capers and other fruits) are found to have anti-depressive effects as they can suppress Monoamine oxidase A [12,13,14].

In view of above evidence that exhibits that medicinal plants have the potential to develop the herbal drug for some of the neurological disorders. Computational methods like Molecular Docking help in understanding the structure and function of bio-molecules and design of structure- based rational drugs. Docking Studies focus on identifying novel compounds from medicinal plants possessing therapeutic properties through binding interactions between ligand and target. In this study, compounds belonging to different medicinal plant categories were studied using Hex 8.0.0 docking software. Natural compounds derived from plants like Capsaicin (CPS), Nordihydrocapsiate (CPT), Avicin-B, Bacoside-A, Lobeline, Lupanine, Apigenin, Rutin, Quercetin, Saligcinnamide, Macluraxanthone-C, Nitidine, Curcumin and Naringenin are subjected to docking simulations against different molecular targets.

Therefore, in the present study, an effort has been made to demonstrate drug likeliness of natural compounds *in silico*, by molecular interaction studies with the targets of few important neurological disorders.

MATERIAL AND METHODS

Hex 8.0.0 was used for docking. In Hex's docking methodology, each molecule is constructed using 3D expansions of orthogonal spherical polar basis functions to incorporate both surface shape and electrostatic charge and potential distributions for accurate results. This feature allows each property to be represented by a vector of coefficients (which are the components of the basic functions). Thus expressions are written for the overlapping of pairs of parameters. This helps to obtain an overall docking score as a function of the six degrees of freedom. The ligand is said to rigid in hex molecular docking software. With suitable scaling factors, this docking score can be interpreted as an interaction energy, which needs to be the least. Hex accepts structure files of protein and DNA molecular structures in PDB-format.

Ligand Preparation

The 3D structures of the 15 plant derived compounds were obtained from Pubchem

(https://pubchem.ncbi.nlm.nih.gov/) in PDB format. PubChem is a consortium of three databases, which are linked within the NCBI's Entrez information retrieval system. The ligands were available in XML, SDF, JSON and ASN formats. Most structures were obtained in XML format. These conformations were used as starting conformations to perform docking. Structures of all 14 ligands used for the study are Apigenin (Figure 1), Quercetin (Figure 2), Rutin (Figure 3), Capsaicin (Figure 4), Lupanine (Figure 5), Saligcinnamide (Figure 6), Bacoside A (Figure 7), Nordihydrocapsiate (Figure 8), Avicin B (Figure 9), Macluraxanthone C (Figure 10), Nitidine (Figure 11), Lobeline (Figure 12), Naringenin (Figure 13), Curcumin (Figure 14).

Target Prediction

Target prediction was done using Swiss Target search. A web server is a part of Swiss Institute of Bioinformatics (SIB). Target of small molecules can be predicted with this site. A selected molecule was screened against a repository of 280000 compounds on more than 2000 targets. It has the ability to predict similarity in 5 different organisms like humans, horse, bovine cattle organisms, house mouse and brown rat. Swiss Target Prediction accurately predicts the targets of biomolecules based on 2D and 3D similarity parameters with known ligands. Swiss Target Prediction is accessible free of charge.

Target Preparation

Once the appropriate targets were found using Swiss Target Prediction, the most effective and fitting target was chosen by performing secondary research through international articles, journals and publications.

The X-ray crystal structures were obtained from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) in PDB format. Targets were downloaded in such a way that heteroatoms like water, ions etc. were not present and they were not complexed with other bioactive molecules. Those structures, which were found complexed with other bioactive molecules, were individually modeled using SWISS MODEL (http://swissmodel.expasy.org/) and Phyre 2. Phyre is typical structure prediction systems software, which uses algorithms, all these systems, can reliably detect up to twice as many remote homologies as standard sequence-profile searching. The SWISS-MODEL template library provides a detailed understanding of quaternary structure and essential ligands and cofactors to allow for building of complete structural models, including their oligomeric structure. The improved SWISS-MODEL makes use of accurate algorithms for selection of the most suitable templates and provides estimates of the expected accuracy of the resulting models. SWISS MODEL uses the FASTA format of protein sequences.

Ligand Visualization

The ligand and target in the PDB format were visualized in Argus Labs. Argus Lab is a molecular modelling, graphics and drug design program for Windows operating systems. Argus Lab can be downloaded and accessed free of charge.

Docking Method

The docking analysis of the given ligands was carried out using Hex 8.0.0. It is a molecular graphics program that calculates and analyzes docking modes of protein and DNA molecules along with protein ligand docking, assuming the ligand is rigid and superposes molecules solely based on 3D structure. The parameters for docking were set as follows:

Correlation type: Shape +Electro

FFT Mode: 3D

Grid dimension: 0.75

Solutions: 5000

Receptor range: 180

Ligand range: 180

Twist range: 360

Distance range: 40

Box size: 10

HEX 8.0.0 docking software, which is an Interactive Molecular Graphics Program for

calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Docking allows predicting the ligand with best E scores and identifying the drug-receptor complex with lowest free energy and thus is an indispensable in drug discovery and drug target interaction studies.

Structures of Studied Natural Compounds

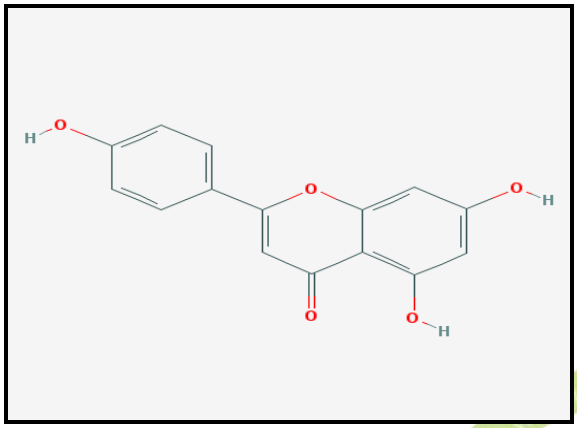

Figure 1: Apigenin

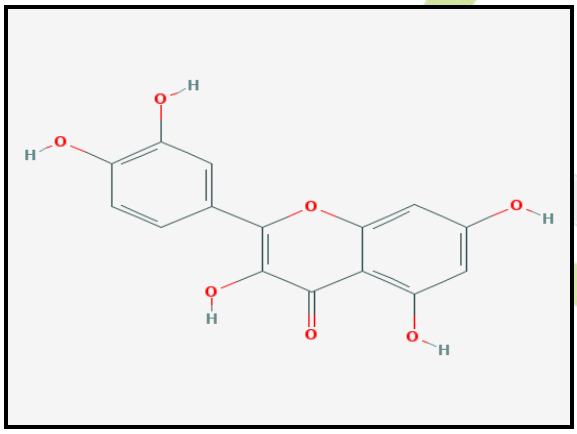

Figure 2: Quercetin

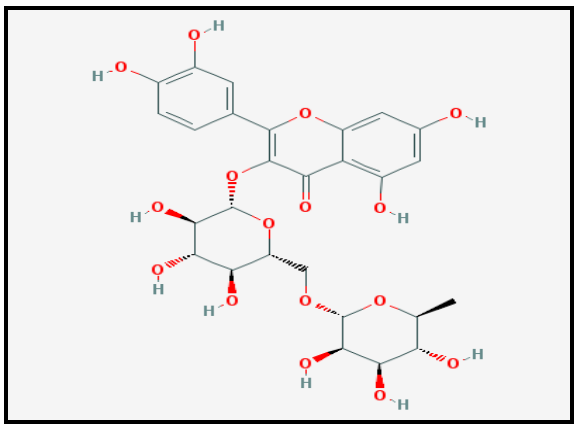

Figure 3: Rutin

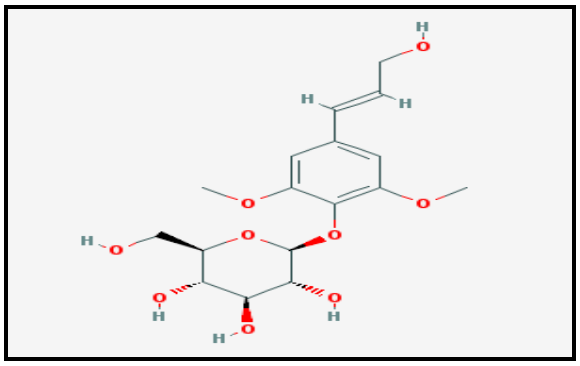

Figure 4: Capsaicin

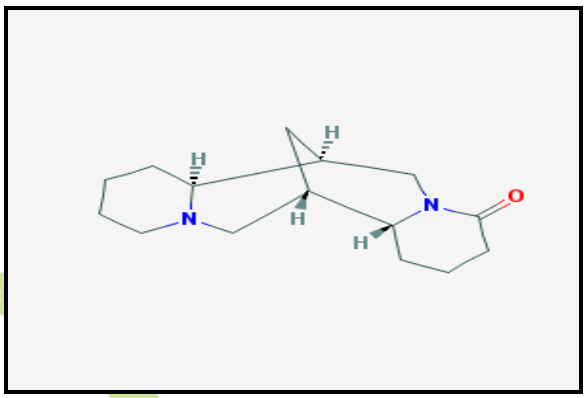

Figure 5: Lupanine

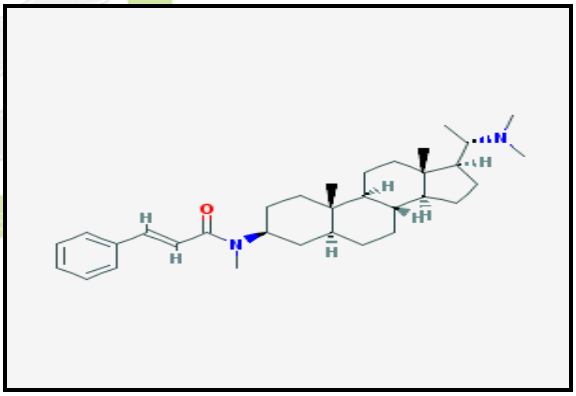

Figure 6: Saligcinnamide

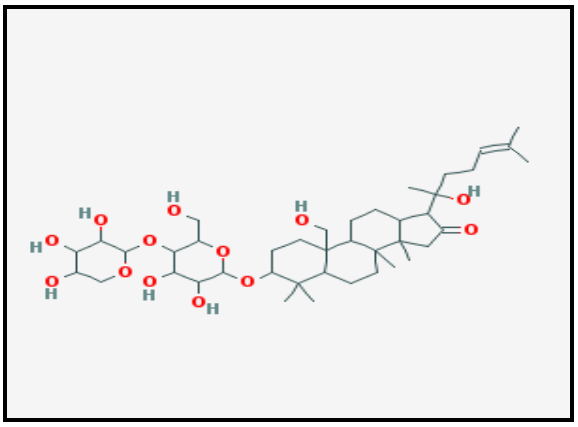

Figure 7: Bacoside A

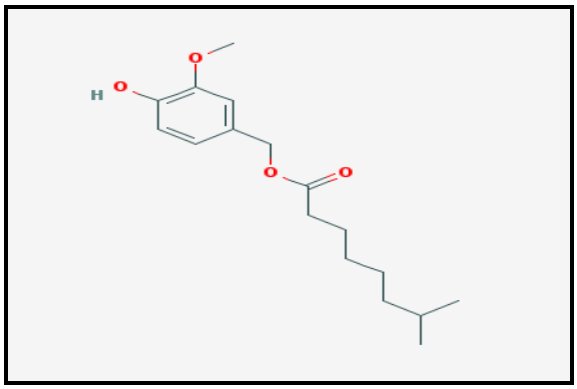

Figure: 8 Nordihydrocapsiate

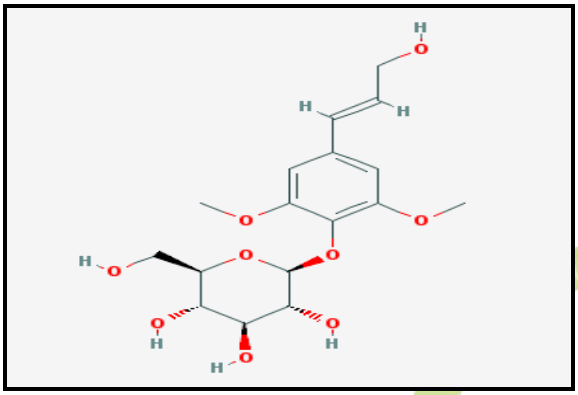

Figure 9: Avicin B

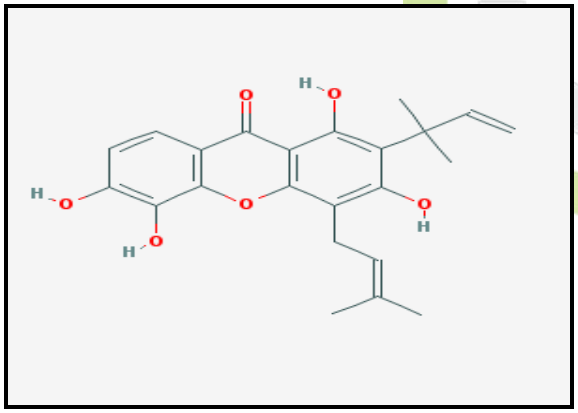

Figure 10: Macluraxanthone C

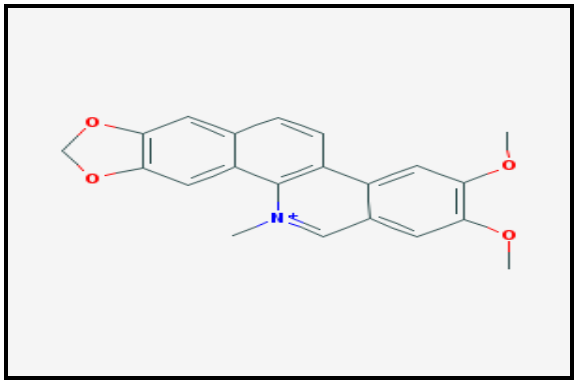

Figure 11: Nitidine

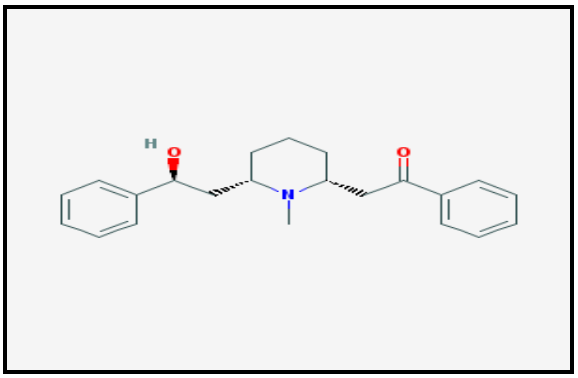

Figure 12: Lobeline

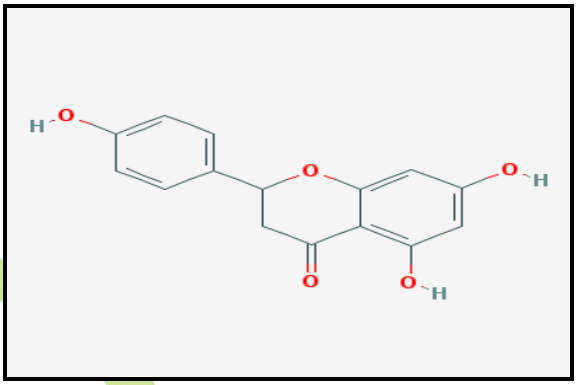

Figure 13: Naringenin

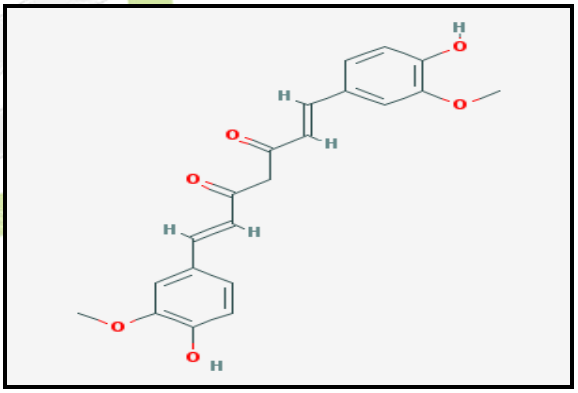

Figure 14: Curcumin

RESULTS AND DISCUSSION

The docking simulation technique was performed using Hex 8.0.0 with 14 plant-derived compounds. Each compound was docked to the same or a different target. The lowest energy docked conformation was selected and taken into account. To compare the docking interactions selected natural compounds were docked against many potential inhibitors in a biochemical pathway for treatment of a particular disease. The results have been summarized in Table 1. In the

screenshots, the natural compounds are represented as dotted structures while their receptors are solid ball structures.

Docking results for binding between Capsaicin and COX-2 showed that Capsaicin inhibits COX-2 well having an E-value of -263.92. Nordihydrocapsiate has a good binding interaction also with the E-value of -274.37. Binding interaction value between Avicin- B and COX-2 is -461.73 (Figure 15). Macluraxanthone C has a low binding energy value of -107.45. Avicin-B binds the best with Cox-2, better than the most known inhibitors of COX-2. COX-2 plays a role in Neuro-inflammation in different neurodegenerative disorders like Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis[14].

It helps in the converting arachidonic acid to prostaglandin (PG) H2, the main precursor for PGE2. Increased levels of PGE2 can result in formation of different reactive oxygen species. PGE2 can also interact with EP receptors and promote the process of neurodegeneration.COX-2 inhibition protects the degeneration of motor neurons and has been shown to prolong survival in mouse studies of Parkinson's Disease and Amyotrophic Lateral Sclerosis[5,14]. Docking Results for binding between Bacoside-A and NF-Kβ in Fig 16 show that Bacoside-A inhibits NF-Kβ well having an E-value of -391.31.

Capsaicin and Nordihydrocapsiate inhibit NF-Kβ with the binding energies of -253.35 and -252.37 respectively. Binding interaction energy value between Avicin-B and NF- Kβ is -1278.23, which is unusually high. Bacoside-A seems to inhibit NF- Kβ the best. NF- Kβ is involved in the expression of certain pro-inflammatory genes and cytokines, chemokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNFα)[16]. Inhibition of NF- Kβ helps in the treatment of neurological disorders like Parkinson's disease[3].

Macluraxanthone C (derived from *Maclura pomifera*) belongs to the family of Xanthones. It has shown to have anti-inflammatory properties. Docking results for binding between Macluraxanthone C and Monoamine oxidase-A showed that Macluraxanthone C inhibits MAO-A with an E-value of -241.32.Monoamine oxidase-A (MAO-A) is one of the main enzymes involved in the metabolism of the neuro-transmitter serotonin (5-hydroxytryptamine).

Increased activity of MAO-A in the brain can contribute to causing different depressive disorders. Macluraxanthone C has a suppressing effect on MAO-A and exhibits the potential to play an essential role in treatment of Major Depressive Disorder (MDD)[11]. Lead compounds like Lobeline (derived from *Lobelia inflata*) and Lupanine (derived from *Lupinus polwbyllus*) are found to have the potential to inhibit Dopamine D2 receptor (Wiert.2014). Docking results for these compounds show that Lobeline inhibits D2 Receptor with E-value of -293.11 and Lupanine has a binding interaction value of -233.27. Saligcinnamide (a steroidal alkaloid derived from *Sarcococca saligna*[10] has binding interaction value of -400.29 against Dopamine D2 Receptor (Fig 18).

This suggests that Saligcinnamide binds exceptionally well with D2 receptor, showing inhibition potential more than present inhibitors.

Dopamine D2 receptor antagonism helps in the treatment of Alzheimer's disease by suppressing Neuro-toxicity and Tau aggregation[7].In addition, inhibition of Dopamine D2 Receptor has shown improvements in symptoms of patients suffering from Schizophrenia[8].

Flavonoids like Curcumin (belonging to *Zingiberaceae*), Narginenin (derived from grapefruits and tomatoes), Nitidine (derived from *Zanthoxylum*) were docked against Acetylcholinestearase. Curcumin inhibits Acetylcholinestearase well with an E-value of -304.19. Narginenin and Nitidine have binding interaction values of -232.41 and -292.36. Saligcinnamide showed inhibition potential as well with the E-Value of -336.14 (Fig 17), the best among all these compounds. Acetyl cholinesterase is involved in the hydrolysis of the neurotransmitter acetylcholine. Inhibition of this enzyme plays a key role in treatment of Alzheimer's disease.[10]

Table 1: Binding Energies of Natural Compounds Docked with Target Molecules

Sr No.	Natural Compounds	Target Molecules	Binding Energy	Disease
1	Capsaicin	NF-Kβ	-256.15	Parkinson's Disease
2	Capsaicin	COX-2	-263.98	Amyotrophic lateral sclerosis (ALS)
3	Nordihydrocapsiate	NF-Kβ	-252.37	Parkinson's Disease
4	Nordihydrocapsiate	COX-2	-274.37	Amyotrophic lateral sclerosism (ALS)
5	Avicin B	COX-2	-552.45	Amyotrophic lateral sclerosis (ALS)
6	Avicin B	NF-Kβ	-1278.23	Parkinson's Disease
7	Bacoside A	NF-Kβ	-391.33	Parkinson's Disease
8	Macluraxanthone C	COX-2	-107.15	Amyotrophic lateral sclerosis (ALS)
9	Macluraxanthone C	MAO-A	-241.32	Major depressive disorder
10	Saligcinnamide	AChE	-336.14	Alzheimer's disease
11	Saligcinnamide	D2 DOPAMINE RECEPTOR	-400.29	Alzheimer's disease Schizophrenia
12	Curcumin	AChE	-304.19	Alzheimer's disease
13	Nitidine	AChE	-292.36	Alzheimer's disease
14	Nitidine	COMT	-251.75	Parkinson's Disease
15	Lobeline	D2 DOPAMINE RECEPTOR	-293.11	Alzheimer's disease Schizophrenia
16	Lupanine	D2 DOPAMINE RECEPTOR	-233.27	Alzheimer's disease Schizophrenia
17	Apigenin	Monoamine oxidase A	-210.60	Major depressive disorder
18	Quercetin	Monoamine oxidase A	-206.08	Major depressive disorder
19	Rutin	Monoamine oxidase A	-245.66	Major depressive disorder
20	Naringenin	AChE	-232.41	Alzheimer's disease

Nitidine is also docked with Catechol-o-methyl transferase. The binding energy value is -251.75 (Fig 19). Catechol-o-methyl transferase metabolizes Levadopa and makes it unavailable for conversion to Dopamine. Inhibition of COMT can thus help in improving effects of Parkinson's disease.

Several reports show that Apigenin, Rutin, Quercetin and Luteolin inhibit Mono amine oxidase A thus preventing the 5-hydroxytryptopan (serotonin) to transform to 5-hydroxyindole acetic acid. Lower the levels of serotonin, greater the gravity of depression. Docking results of binding between Apigenin and MAO-A in shows that Apigenin inhibits Monoamine Oxidase-A well having an E score of -210.06. Docking analysis shows E total values of Apigenin, Quercetin, Luteolin and Rutin to be -210.06,-206.08,-96.15 and -245.66 when bound with the target Mono Amine Oxidase-A It can be seen while Apigenin and Quercetin show similar binding properties with Monoamine oxidase A, Rutin binds the best with MAO-A (Fig 20)[12,13,14].

The given paper advocates the use of computational analysis and importance of medicinal compounds for the treatment of complex neurological disorders. Computer aided drug design has caught attention of many scientists lately as it allows fast screening of ligands and drug targets by finding the best possible binding mode and the stability of the complex. This cuts down both the speed and costs of drug discovery and development research. Thus, focus has changed from traditional methods to genomics-and proteomics-based drug research strategies.

Hex 8.0.0 has been used for docking various novel pharmacological compounds with appropriate targets in this study. Phytochemicals from broad categories like triterpenoids, flavonoids, lead compounds and capsaicinoids were selected. Compounds like Capsaicin, Nordihydrocapsiate, Avicin B, Bacoside A, Macluraxanthone C, Saligcinnamide, Curcumin, Nitidine, Lobeline, Lupanine, Apigenin, Quercetin and Rutin have been docked against various important biological targets, which play a vital role in cell signaling and biochemical pathways of Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Schizophrenia and major depressive disorder. The docking results of most compounds with targets exceeded expectations with range of E score of inhibitory interactions being -250 to -300.The interactions in which the drug target was activated by the ligand (phytocompounds) had E scores of the range -1000 to -1400.Nordihydrocapsiate, Capsaicin and Avicin B show best results for treatment of ALS, Parkinson's and Alzheimer's disease. Bacoside A proves to be the best inhibitor of NF-Kβ and thus treatment of Parkinson's disease. Saligcinnamide binds well with Dopamine D2 receptor and plays an antagonistic role in Alzheimer's disease pathway. Rutin is the best inhibitor of MAO-A and hence ameliorates depression. These values validate that these phytocompounds form stable complexes with their respective targets as they have a large negative E score values. Large negative E scores show that the complex formation is an exothermic process and the energy possessed by the ligand-target complex is low thus making it stable. Medicinal plants being cheaper, more accessible and more effective could serve as futuristic pharmacological agents. Patients thus leading to lesser adverse drug reactions as opposed to conventional drugs more easily tolerate them. They also cross the blood brain barrier easily. Hence, medicinal plants can be the model drugs for the future and can be the foundation for designing synthetic drugs.

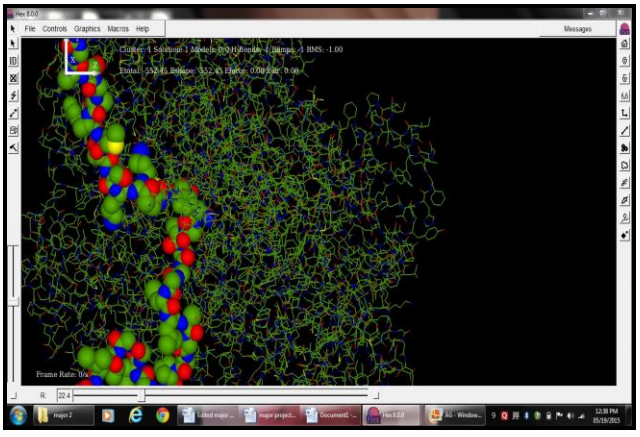

Figure 15: Screenshot showing Docking of Avicin B with Cox-2

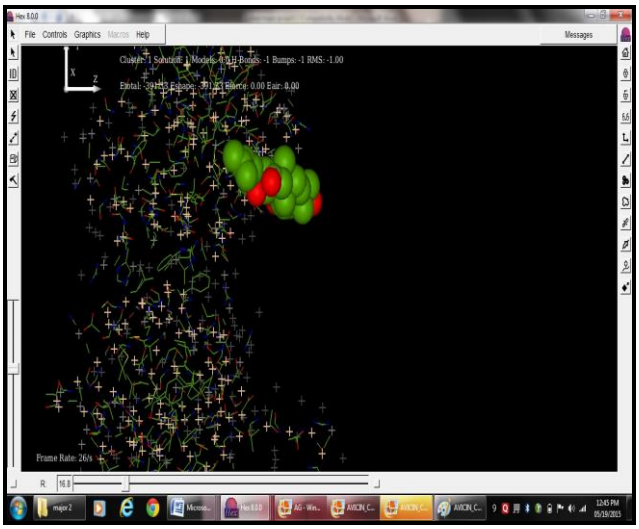

Figure 16: Screenshot showing Docking of Bacoside A with NF-Kβ

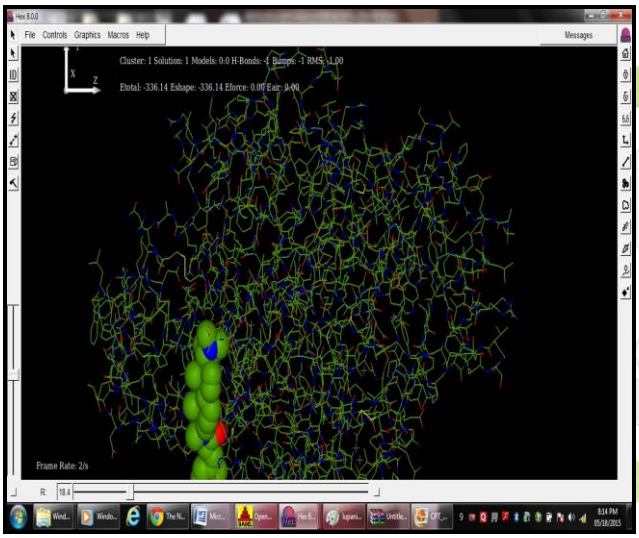

Figure 17: Screenshot showing Docking of Saligcinnamide with Ache

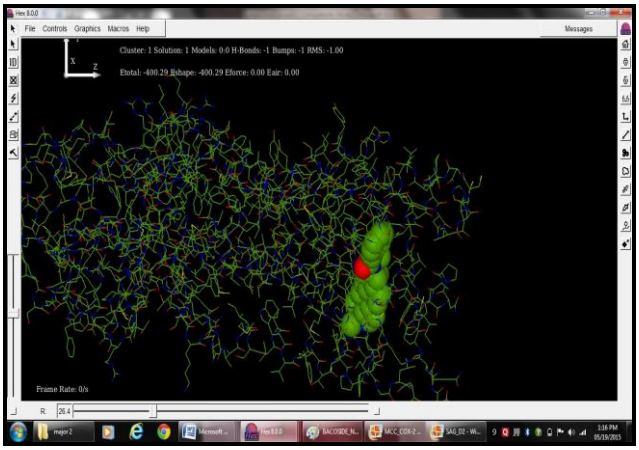

Figure 18: Screenshot showing docking of Saligcinnamide with D2 receptor

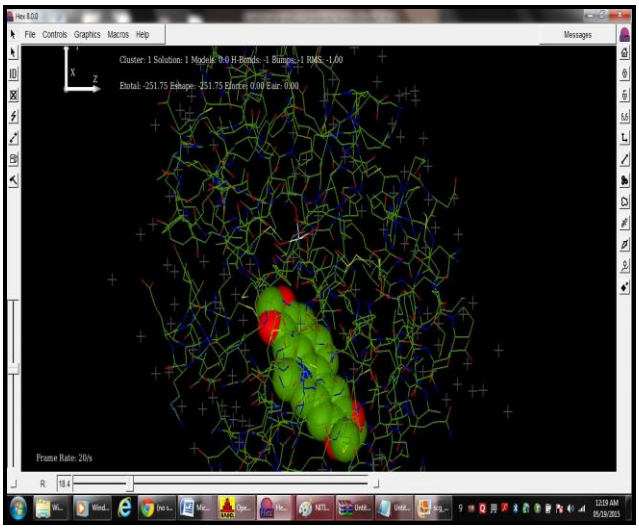

Figure 19: Screenshot showing Docking of Nitidine with COMT

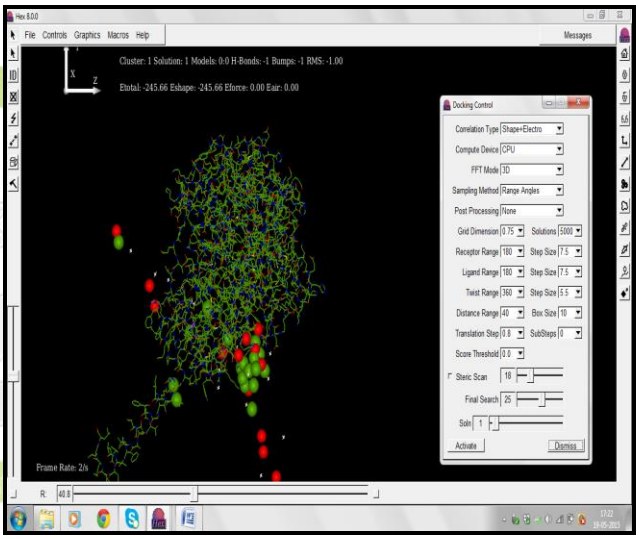

Figure 20: Docking of Rutin with MAO-A

CONCLUSION

Neurological disorders pose a great threat to the human population. The ambiguity in the mechanisms involved in the degeneration of neurons increases the danger. The *in-silico* method adopted in the present study helped in identifying the natural compounds for the treatment of neurological disorders. This knowledge is necessary for the development of novel drugs for the treatment of Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Schizophrenia and Major Depressive Disorder. We conclude from this pilot study that this piece of work might give a new

insight for the new drug development against these Neurological disorders.

ACKNOWLEDGEMENTS

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